

Report Date: 22 January 2007

Report Type: Final report

Dates covered: 1 November 2005 - 1 November 2007

Title:

Assessment of Zebra Mussel (*Dreissena polymorpha*) Infestation Risk Using GIS for Water Basins in the North-West Bulgaria

Contract No. N62558-05-P-0476

Project No. R&D 1021-EN-01

Authors:

Teodora A. Trichkova¹, Alexander A. Kotsev², Anton Popov², Mladen T. Zivkov¹,
Dimitar S. Kozuharov³, Ivan S. Botev¹, Zdravko K. Hubenov¹, Stelian Dimitrov²,
Svetoslav D. Cheshmedjiev

Performing Organizations:

¹ Institute of Zoology, Bulgarian Academy of Sciences; 1 Tsar Osvoboditel Blvd.,
Sofia 1000, Bulgaria

² Faculty of Geology and Geography, Sofia University; 15 Tsar Osvoboditel Blvd.,
Sofia 1504, Bulgaria

³ Faculty of Biology, Sofia University; 8 Dragan Tsankov Blvd., Sofia 1164, Bulgaria

Sponsoring Agency:

U.S. Army Engineer Research and Development Center's International Research
Office

Distribution Statement: Approved for public release, distribution unlimited

Contact Information:

Teodora Trichkova

Institute of Zoology, Bulgarian Academy of Sciences

1 Tsar Osvoboditel Blvd., Sofia 1000, Bulgaria

Tel.: +359-2-9884708

Fax: +359-2-9882897

E-mail: trichkova@zoology.bas.bg;

trichkova@gmail.com

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 22 JAN 2007		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE Assessment of Zebra Mussel (Dreissena Polymorpha) Infestation Risk Using GIS for Water Basins in the North-West Bulgaria				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Institute of Zoology Bulgarian Academy of Sciences 1 Tsar Osvoboditel Blvd Sofia 1000, Bulgaria				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES The original document contains color images.					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT SAR	18. NUMBER OF PAGES 54	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

Abstract

In Bulgaria, zebra mussel (*Dreissena polymorpha*) occurred originally in the Danube River and the Black Sea coastal lakes and rivers. During the last 10 years a rapid spread of this species in the inland water bodies has been observed. Two of the reservoirs in the North-West Bulgaria have been reported as infested. The goal of the present study was to determine the risk of zebra mussel infestation in this region by combining available biological and environmental data and by subsequent processing and analyzing of these data using Geographic Information System (GIS). This will be the first step in developing an action plan for vulnerable regions in Bulgaria.

The evaluation of physical and chemical characteristics of water bodies and their suitability to the zebra mussel requirements was made based on existing data from monitored river stations, spatial data and reservoir field survey. Fifteen reservoirs were sampled to determine which physicochemical variables explain the occurrence and abundance of zebra mussel. The principal component analysis used showed that the first two principal components related mainly to calcium concentration, pH, electroconductivity and Secchi disk transparency, explained the majority of total variance of data. pH, calcium concentration and dissolved oxygen were selected as habitat suitability parameters, and corresponding tolerance ranges based on the environmental requirements of zebra mussels during the larval and early growth stages, were defined. These parameters were divided into three main categories that reflected the infestation potential of zebra mussel: Low, Moderate and High. After all spatial and attributive data were collected, they were processed and analyzed in a GIS environment, which included the GIS database development, spatial and geostatistical interpolation of limiting factors, reclassification and overlay.

The results showed that based on water chemistry, 63.81% of the territory can be classified with high potential, 15.45% - with moderate potential, and 20.74% - with low potential for zebra mussel infestation. Additional factors in reservoirs contributing to the increased risk of infestation are large surface area (over 90 ha) and depth, high substrate diversity, moderate amount of nutrients and easy accessibility to human users. As potential zebra mussel dispersal mechanisms were identified the direct waterway connections with the Danube, as well as transport of larvae or adult individuals with fishing equipment, boats and fish stocking material from the Danube and fish farms nearby.

Key words: Aquatic invasive species, *Dreissena polymorpha*, infestation potential, reservoirs, North-West Bulgaria, risk assessment, GIS

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1. Introduction

The invasion of non-indigenous species is now considered to be one of the most serious problems facing native ecosystems (Lodge, 1993; Simberloff, 1996; Wilcove et al., 1998) and the invaders are viewed as a significant component of global change (Vitousek et al., 1996). They have received increasing attention also for their considerable economic and social impacts (Pimentel et al., 2000, 2001).

A typical invasive species with great potential to cause ecological and economic damages is the freshwater bivalve mollusk zebra mussel (*Dreissena polymorpha*) (Ludyanskiy et al., 1993; Nalepa & Schloesser, 1993). This is a filter feeding, fast reproducing species (females producing up to one-half million eggs per year) with two stages of development - planktonic veliger larvae and attached adult form. With the help of strong byssal threads adult zebra mussels attach to hard submerged substrates or often to each other creating large colonies. When with high abundance, zebra mussels can dramatically change the ecology of infested water bodies by reducing plankton populations (MacIsaac et al. 1995, Caraco et al. 1997, Bastviken et al. 1998, Pace et al. 1998) or causing shift in their diet (Maguire & Grey 2006), and by adversely impacting benthic invertebrate communities and fish populations (Mackie 1991, Ricciardi et al. 1996, Nalepa et al. 1996, 2001, Schloesser et al. 1996, Karatayev et al. 1997, Burlakova et al. 2000, Strayer et al. 2004). At the same time they can interfere with vital water supply intakes and navigation structures and thus affect thermoelectric and nuclear power plants, drinking water treatment plants and various industries (Clarke 1952, Erben et al. 2000).

The zebra mussel is a Ponto-Caspian relict species native to the drainage basins of the Black, Azov, Caspian and Aral Seas (Golikov & Starobogatov 1968, Skarlato & Starobogatov 1972, Valkanov et al. 1978, Marinov 1990). In Bulgaria, zebra mussel was first reported for the Danube River by Kreglinger (1870). Later, its occurrence was recorded in the Danube and its tributaries, as well as in the Black Sea coastal lakes and rivers (Kobelt 1897-1898, Wohlberedt 1911, Hesse 1914, Drensky 1947, Petrbock 1947, Valkanov 1957, Russev et al. 1994, Angelov 2000). Fossil specimens were recorded in Pleistocene and Holocene sediments from the North-West Bulgaria, along the Danube, from several Black Sea coastal lakes and parts of the Bulgarian Black Sea Shelf zone (Shopov 1979, 1984, Hrishev & Shopov 1979, Liutskanov 1981). Recently, a rapid spread of zebra mussel in the Bulgarian inland water bodies has been observed. It was reported from the Reservoirs Ovcharitsa, Zhrebchevo and Sopot (Central and South-East Bulgaria), as well as from the Lake Chepintsi (West Bulgaria) (Hubenov 2005). Most recent records were from the Reservoirs Pyassachnik, Ticha and Malko Sharkovo (Central and East Bulgaria) (Black Sea and East-Aegean Sea Basin Directorates). The reservoirs in the North-West Bulgaria are considered of great vulnerability to infestation by zebra mussel because of their proximity to the Danube River. Two of them - Reservoirs Rabisha and Ogosta have already been reported as infested (Hubenov 2002, 2005). The zebra mussel infestations had strong impact on the infested reservoirs. As a result of infestation of the Cooling-Reservoir Ovcharitsa, the Maritsa-East 2 Thermoelectric Power Plant has suffered continuous problems, such as: clogging of cooling system pipes, screens, pumps and other facilities, as well as enormous increase in corrosion of metal surfaces (Hubenov 2002). Damages on irrigation and fish cage facilities were reported in the Reservoirs Ovcharitsa, Ogosta and Rabisha.

The first and most cost-effective option when dealing with invasive species is prevention. Assessment of potential risk of zebra mussel infestations would help water managers and responsible authorities to plan for and to prevent major adverse

impacts from zebra mussels when they appear in a water body. Risk assessment methods have been widely used recently when dealing with issues concerning zebra mussel and other invasive species (Johnson et al., 2001, Brooks & Shlueter, 2004). These efforts include developing classification schemes to predict invasiveness, identifying sources or pathways of introduction and susceptible resources and characterizing the potential biological consequences of establishment and spread. A common method of most studies on zebra mussel is comparing limiting conditions for the species to environmental data (climatology, geology, water chemistry, etc.) (Strayer, 1991, Ramcharan et al., 1992, Schmidt & Hirsch, 1993, Strayer & Smith, 1993, Mellina & Rasmussen, 1994, Whittier et al., 1995, Hayward & Estevez, 1997, Lewis et al., 1997, Cohen & Weinstein, 1998a,b, Kozlowski et al., 2002, Jones & Ricciardi, 2005). Geographic Information System (GIS) technology is increasingly being used as risk assessment tool (Lavakare, 2005). Geographic Information Systems provide an effective platform for designing an integrated database for assessing risk and developing risk reduction strategies (Amoako-Atta & Hicks, 2004). Since the data on zebra mussel infestation risk are spatial dependant they can easily be stored, processed and analyzed in GIS databases. Neary & Leach (1992) evaluated the zebra mussel colonization risk with a GIS and mapped the potential spread of the species in Ontario by matching mussel tolerances to pH and Ca to data from 6 151 lakes. Other authors also use GIS to predict the spatial distribution of zebra mussel (Doll, 1998; Koutnik & Padilla, 1994; Haltuch et al., 2000).

The goal of the present project was to determine the risk of zebra mussel infestation in water bodies in the North-West region of Bulgaria by combining available biological and environmental data and by subsequent processing and analyzing of these data using Geographic Information System. Assessing the risk of infestation will be the first step in developing an action plan in this and other vulnerable regions in Bulgaria.

2. Study Area

The study area included the North-West part of Bulgaria. Its territory is 9105,38 km², which is approximately 8.2 % of the territory of Bulgaria. It is characterized with a diverse landscapes and geographic conditions. 18.34 % of the region is underlain by carbonate rocks, karstified to a certain extent. The area is constructed of three structural entities – the Danubian plain, transitional “Predbalkan” and the Balkan Mountains. The variation in elevation is over 1800 m.

The study area hydrographic system includes 13 main river catchments and 280 big and small reservoirs affiliated to the Danube River basin (Fig. 1). These are the drainage areas of the following rivers: Timok (with 2 reservoirs), Deleinska (with 4 reservoirs), Topolovets (6 reservoirs), Voinishka (9 reservoirs), Vidbol (4 reservoirs), Archar (6 reservoirs), Skomlya (2 reservoirs), Lom (26 reservoirs), Tsibritsa (38 reservoirs) and Ogosta (183 reservoirs). The reservoirs in the region are used mainly for irrigation, aquaculture, commercial and recreational fishing. A small number of them are used for electricity production and drinking water supply.

3. Material and Methods

3.1. Data Collection

Various sources were used for collecting data. Data about physiological tolerances of adult and larvae zebra mussel were retrieved from the literature. Data about the

physical and chemical characteristics of the water bodies in the region, as well as about fish stocking were collected from:

- Existing spatial data and databases compiled from stations monitored by Regional Environmental, Meteorological and Hydrological Laboratories
 - Ministry of Environment and Waters of Bulgaria
 - National Institute of Meteorology and Hydrology, Bulgarian Academy of Sciences
 - National Agency of Fisheries and Aquaculture, Ministry of Agriculture and Forestry
 - National Irrigation System Company
- EEA (European Environmental Agency), NASA SRTM
- Digitalization of paper maps
- Field survey

The analysis of depth, substrate type and Secchi disk transparency as parameters of importance to zebra mussel physiological and habitat requirements were based on survey and published data. The analysis of water temperature, pH, dissolved oxygen and calcium concentration were based on:

- survey data
- published data
- spatial data from 17 hydrological stations (1975) (min, max, avg, std)
- linearly referenced data - 6-year period (2000-2005) from 13 hydrological stations at the rivers Voinishka, Archar, Lom, Tsibritsa and Ogosta
- data for 9-year period (1998-2006) from one hydrological station at the Ogosta Reservoir
- data from 24 stations at the Danube River for the period 1990-2005
- geology.

3.2. Field Survey

Fifteen reservoirs in the study region were sampled to determine which physicochemical variables explain the occurrence and abundance of zebra mussel (Fig. 2, Table 1). The reservoirs were selected for field sampling according to the following criteria:

- River catchment – the most river catchments be represented
- Size - surface area above 20 ha
- Reservoirs probably infested (Rabisha, Ogosta, Mishkovets); reservoirs with possible connection with infested ones (Oshane), and proximity to the Danube (Lipnitsa, Kovachitsa, Asparuhov Val).
- Economic importance - reservoirs used for drinking water supply (Srechenska Bara), electricity production (Ogosta, Christo Smirnenski, Poletkovtsi), aquaculture (Drenovets, Lipnitsa), and fishery.

Three of the reservoirs have smaller surface area (Table 1), but were included in the study because of their importance according to the other criteria. The Oshane Reservoir is connected with the infested Rabisha Reservoir by an artificial channel. The Skomplya Reservoir represents the Skomplya River basin. The Mishkovets Reservoir was reported as probably infested by local fishery authorities.

Five of the reservoirs were first sampled in April 2006. Then all fifteen reservoirs were sampled in the period 28 August – 07 September 2006 (Fig. 2). The number of sampling sites in each reservoir is shown in Table 2. Maximum possible sampling sites in a reservoir were selected according to their surface area and depth. The geographic coordinates of the sampling sites and the dam of every reservoir were

determined by GPS receivers Garmin 72 WAAS and Garmin Map 60 CSX tuned on WGS 1984 datum.

At every sampling site, water chemistry, benthic and plankton samples were taken. Besides, benthic samples were taken from additional sampling sites (control samples) to check for the occurrence of adult zebra mussels. In every reservoir, the dam, water intake tower, irrigation facilities and accessible banks were checked for the presence of zebra mussel shells, colonies or fouling.

The quantitative benthic samples were collected with a Petersen bottom sampler of medium size (17.0x16.5 cm) at deeper sites and Surber sampler frame (35x30cm) at shallow sites. The plankton samples were collected with qualitative Apstein and quantitative Judau nets of 38 μ m mesh size. The adult zebra mussels found and plankton samples were fixed in 4% formalin. Transparency was measured with a Secchi disk. The water samples were collected with water sampler of Hydrobios PVS 436 302. Water temperature, dissolved oxygen, oxygen saturation, pH and electroconductivity were measured using portable Oxygen, pH and Conductivity meters Schott GMBH. One liter water samples were taken in plastic bottles and transported to the laboratory in a cooler with ice for determining calcium and bicarbonate concentrations.

At the laboratory, calcium concentration was determined using volumetric method with Na₂EDTA solution with murexide indicator. Bicarbonate concentration was determined by titration with HCl solution and methylorange as end point indicator (Golterman & Clymo 1970, Höll et al. 1970). The plankton samples were studied for presence of veliger larvae, and benthic samples – for presence and abundance of adult zebra mussels. The zebra mussels found were sorted and measured. Measurements were made with a caliper, micrometric scale of stereomicroscopes Leica GZ 6 and MBC – 9 and electronic balance to the nearest 0.1 mm and 0.1 g, respectively. Absolute abundance and total biomass were calculated per square meter.

3.3. Assessment Methods

3.3.1. Statistical Methods

For the purposes of the statistical analysis we used data from one sampling site at each reservoir. In the reservoirs, where more than one sampling site existed, the sites were selected randomly. Data from April and data from August-September were analyzed separately. Ordination technique based on principal component analysis (PCA) was used to summarise the major patterns of variation within the data obtained. Ordination was implemented by the computer program CANOCO 4.0 (Ter Braak & Šmilauer 1998). The program STATISTICA (StatSoft, Inc. 2001) was used to analyze correlations and to describe the distribution of important environmental variables in different water bodies.

3.3.2. Selection of physicochemical parameters and suitability criteria

Based on the results of the principal component analysis and review of available information, habitat parameters important for the larval and early stages of zebra mussel survival and growth were selected and suitability criteria defined. These parameters were divided into three main categories that reflected the infestation potential of zebra mussel: High, Moderate and Low Potential. The parameters that were in the range known to support reproducing zebra mussel populations were included in the category “High”; these in the range in which zebra mussel larvae are

known to survive but not well - in “Moderate”; and the parameters that were outside the range in which zebra mussel larvae are known to occur - in “Low”.

3.3.3. GIS Methods

Analysis of available input information

All available information sources and different kind of input data were analyzed in order to select the spatial phenomena and to define their geometric and attributive characteristics. The following table was made and used as reference for the organization and entering of data:

Object	Attributes (number of fields, object name, type of the data, etc.)	Geometry (point, line, polygon or raster)
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Preparation of a GIS database

The building of the GIS database included 2 stages: 1) Creation of digital layers from analogue maps (digitizing); and 2) Organization of all layers into an object-oriented topologically correct geodatabase. The GIS database developed contained the following feature classes:

- Contours (40 meter intervals) digitized from paper maps, Scale 1:100 000;
- Hydrologically and geomorphologically consistent digital elevation model (DEM), with cell size of 50 meters, constructed by using topogrid function from the contours, points and hydrographic polyline features;
- Watersheds – delimited through derivatives (flow direction and flow accumulation) of the sinkless digital elevation model developed;
- Hydrography (topologically sound rivers and lakes), originating from paper maps in scale 1:50 000;
- Settlements;
- Roads;
- Geological layer - constructed from 1:50 000 geological paper maps, needed to estimate calcium quantities within the separate watersheds through spatial statistical analysis;
- Soil layer- constructed from 1:100 000 soil paper map;
- Land cover- derived and processed based on the EU CORINE land-cover (CLC2000) project.

Each of these layers was properly geometrically registered with other layers. Paper maps were the primary source of input spatial information for developing the GIS database. They were projected in plain through the country’s former national coordinate system of 1970. All scanned paper maps were georeferenced and converted into Universal Transverse Mercator (UTM) projection by using the WGS1984 spheroid, which is an industry standard for geographic information.

GIS database processing

- a. Post-processing of existing spatial data
 - AGREE reconditioning algorithm was applied for the DEM generated to ensure the integration between data about elevation and hydrography.

- ArcHydro geodatabase data model was applied for all geographic layers, consisting hydrologic oriented information by using the ArcHydro Tools extension for ArcGIS 9.1.
 - Attributes – appropriate coding and additional fields were added through the HydroJunctions, HydroEdges and Catchments in the ArcHydro database, in order to describe their interrelation, function, type, direction of flow, as well as some metric characteristics like length.
- b. Linear referencing – A linear referencing algorithm was applied to capture information about the locations of hydrometric stations. Data about the chemical content of water were joined to the point features through a common composite code.

Spatial interpolation of limiting factors

pH

Data on pH consisted of 41 points. The interpolation procedures were based on mean values for the period April-September as input information. Inverse Distance Weighted (IDW) interpolation technique, which algorithm is part of the ArcGIS 9.2 Geostatistical analysis extension, was used. To predict a value for any unmeasured geographical location, the IDW technique uses the measured values surrounding the prediction location. Values closest to the prediction location will have more influence on the predicted value than points which are farther away. Thus, IDW assumes that each measured point has a local influence that diminishes with the increase of distance. It weights the points closer to the prediction location greater than those farther away, hence the name inverse distance weighted.

The optimal weighting power (p) value was determined by minimizing the root mean square error (RMS) of the prediction (Fig. 3). The RMS is the descriptive statistics that is calculated from cross-validation of measured and estimated values. With cross-validation, each measured point is removed and compared to the predicted value for the same location. The RMS is a summary statistics quantifying the error of the prediction surface.

The other important factor determined with IDW interpolation was the search radius, or the extent to which each measurement point has certain influence on the prediction. It is dependent on the number of observations, and therefore, because of the limited number of pH observations available for the territory of concern (41), a higher search radius (neighborhood) was used.

Calcium concentration

The spatial characteristics of the hydrometric stations were used to model the distribution of calcium within the separated catchments / sub-watersheds. Thus, in a continuous (indiscrete) way, as reliable as possible estimation of chemical content in water was achieved. Various interpolation techniques, such as: krigging, other geostatistical methods, IDW, spline, cokrigging, global & local polynomial, etc., were tested. A combination between geology (limestone rock formation spatial distribution, Fig. 4) and the point observations was approbated for the needs of calcium interpolation. Point features were also used to calibrate and determine the efficiency of the interpolation result by comparing predicted/estimated values. Ordinary krigging turned out to be the most precise interpolator for calcium distribution, in terms of root

mean square error (RMS) of predicted vs measured points – 10.63. The correlation function between prediction and observation is illustrated on Fig. 5.

Dissolved oxygen

The dissolved oxygen was estimated based on catchment elevation as a proxy of atmospheric pressure and average slope. The colder the water, the more oxygen can be dissolved. Therefore, dissolved oxygen concentrations at a location are usually higher in winter than in summer, and they are higher at lower altitudes. There is no statistically significant correlation coefficient between catchment elevation and annual average water temperature (only 33,2%). On the other hand, for extreme temperatures, there should be a significant relation between dissolved oxygen and fresh water temperatures (Fig. 6), but it is hard to prove it due to the limited data available for the area of concern. The second factor chosen was slope, since oxygen is more easily dissolved in turbulent water than in slow flowing water.

Reclassification

Reclassification is a GIS-based technique for generalization, which is used to re-assign values in input set of raster layers to create a new output raster data. Reclassification changes the values of input cells on a cell-by-cell basis within the area under analysis. This reclassification technique is commonly used in converting values from one measurement scale to another, always in a direction from a ‘stronger’ to a ‘weaker’ scale (from interval and ratio scale data into ordinal ranking). The process of reclassification always leads to the loss of information (detail), but it creates a common semantic scheme for combining (overlying) various information, such as the data on pH, calcium and dissolved oxygen in our study. Data about the abovementioned raster layers were reclassified according to their relation to the zebra mussel infestation potential in ‘High’, ‘Moderate’, and ‘Low’ categories based on selected suitability ranges.

Overlay

The ability to search for locations of objects or phenomena, which could be characterized through combination (“Overlay”) of factors, is considered as one of the basic GIS functions. Weighted overlay technique was used. This is a technique for applying a common scale of values to diverse and dissimilar input to create an integrated analysis. It is used in solving geographic problems which require analysis of many different factors. Very often, the factors in the analysis are not equally important. The Weighted Overlay process allows taking all issues into consideration. It reclassifies values in the input rasters onto a common evaluation scale of suitability or preference, risk, or some similarly unifying scale. The input rasters are weighted by importance and added to produce an output raster. The steps performed are summarized below:

- A numeric evaluation scale was chosen. Values at one end of the scale represented one extreme of suitability (or other criterion); values at the other end represented the other extreme.
- The cell values for each input raster in the analysis were assigned values from the evaluation scale and reclassified to these values. This makes it possible to perform arithmetic operations on the rasters that originally held dissimilar types of values.

- Each input raster was weighted, or assigned a percent influence based on its importance to the model. The total influence for all rasters equaled 100 percent.
- The cell values of each input raster were multiplied by the rasters' weights.
- The resulting cell values were added to produce the output raster.

The data reclassified in the above steps were afterwards combined using weighted overlay to obtain the needed integral information about zebra mussel infestation potential. With weighted overlay each raster was assigned with a percentage according to its influence in the overall analysis. The separate data layers were weighted as follows:

- Calcium – 40 %
- pH – 40 %
- Dissolved oxygen – 20 %.

4. Results and Discussion

4.1. Occurrence and abundance of zebra mussels in relation to physicochemical factors in the North-West Bulgaria

The results from the field survey were summarized in Table 3. We found three infested reservoirs (Ogosta, Rabisha and Kovachitsa) and two reservoirs with evidence of previous infestation (Drenovets and Asparuhov Val).

Live adult forms and plankton veliger larvae of zebra mussel occurred in the Reservoirs Rabisha and Ogosta. The species had established reproducing populations and adapted successfully to environmental conditions in these reservoirs. The degree of infestation in the Rabisha Reservoir was higher. In the two sampling periods, the mean values of absolute abundance and total biomass of the Rabisha population were much higher than the Ogosta population (Table 3).

In three of the reservoirs, only shells of zebra mussels without live specimens were recorded in the bottom samples. The Drenovets Reservoir was sampled in April and September. Shells of zebra mussels in a comparatively small number (76 shells/m²) were found at one out of three sampling sites only in September. Veliger larvae were not found in the plankton. In the Reservoir Asparuhov Val, a large number of zebra mussel shells were found in the bottom sample (12 630 shells/m²). Moreover, large amounts of shells covered the littoral zone near the dam as well as the entire shore. However, no veliger larvae were found in the plankton samples. Comparatively large number of shells was recorded also in the Kovachitsa Reservoir (2520 shells/m²). They were located mainly in the littoral zone near the dam. Though, there were no live adult specimens recorded in the bottom samples from this reservoir, single live specimens were found at the shore and veliger larvae were recorded in the plankton samples.

In the other 10 reservoirs no adults, larvae or shells of zebra mussel were recorded.

The relation of zebra mussel occurrence and abundance to some of the environmental variables was first studied in five of the reservoirs sampled in April 2006. Principal component analysis (PCA) was used to summarize the major patterns of variation and the results were presented as a PCA correlation biplot (Fig. 7). The first two principal components ($\lambda_1=0.511$, $\lambda_2=0.259$) together explained 77% of total variance of the data. The first axis contrasted infested reservoirs (Ogosta and Rabisha) with high values of Secchi disk transparency (plotted top and bottom on the right of

the diagram) with non-infested reservoirs (Drenovets, Kula and Poletkovtsi) with high values of calcium and bicarbonate concentrations and bigger depths of sampling (plotted on the left of the diagram). Axis 2 reflected the surface area - pH gradient. It separated the more alkaline reservoirs Rabisha, Drenovets and Kula with pH>8 (plotted bottom right and left of the diagram) from the reservoirs Ogosta and Poletkovtsi with pH<8 (plotted top right and left of the diagram.). It also separated the Ogosta Reservoir with the biggest surface area from the other reservoirs. The zebra mussel abundance showed a statistically significant strong positive correlation with transparency (1.00, $P<0.05$) and negative one with calcium concentration (-0.91, $P<0.05$).

The PCA analysis was further developed including data from all the 15 reservoirs sampled in August-September 2006. Electroconductivity (EC) was used as a proxy for nutrient load. The results of the PCA are presented on Fig. 8. The first two principal components ($\lambda_1=0.462$, $\lambda_2=0.183$) explained cumulatively 64.5% of total variance. They reflected two gradients. The first gradient was related to pH-alkalinity and contrasted more alkaline and rich in nutrients reservoirs (with high values of pH, calcium, bicarbonates and electroconductivity) – Lipnitsa, Asparuhov Val, Kovachitsa (plotted top on the left of the diagram) with the lower in nutrients reservoirs with low values of calcium, bicarbonates and electroconductivity – Rabisha, Ogosta, Srechenska Bara (plotted top and bottom on the right of the diagram). The second gradient run from top right to bottom left of the diagram and was related to transparency, zebra mussel abundance and number of stockings. It separated the reservoirs with high abundance of zebra mussel and high values of Secchi disk transparency – Ogosta and Rabisha (plotted top on the right of the diagram) from the other reservoirs with low value of transparency and without live zebra mussels. The zebra mussel abundance showed a statistically significant positive correlations with transparency (0.74, $P<0.05$) and number of stockings (0.73, $P<0.05$).

4.2. Review of physicochemical parameters

Many authors studied the potential distribution of zebra mussel in different regions based on comparison of species physiological and ecological requirements with a variety of environmental factors, including climatic characteristics, geology, water chemistry, etc. Most frequently used and considered as most important for the survival, growth and reproduction of zebra mussel were the following physicochemical parameters:

- temperature (Strayer, 1991, Whittier et al., 1995, Hayward & Estevez, 1997, Cohen & Weinstein, 1998a,b);
- pH (Neary & Leach, 1992, Ramcharan et al., 1992, Koutnik & Padilla, 1994, Whittier et al., 1995, Hayward & Estevez, 1997, Cohen & Weinstein, 1998a,b, Kozlowski et al., 2002);
- dissolved oxygen (Whittier et al., 1995, Hayward & Estevez, 1997, Cohen & Weinstein, 1998a,b, Kozlowski et al., 2002);
- calcium concentration (Neary & Leach, 1992, Ramcharan et al., 1992, Murray et al., 1993, Koutnik & Padilla, 1994, Mellina & Rasmussen, 1994, Whittier et al., 1995, Hayward & Estevez, 1997, Kozlowski et al., 2002, Jones & Ricciardi, 2005);
- substrate size (Mellina & Rasmussen, 1994, Whittier et al., 1995, Hayward & Estevez, 1997, Jones & Ricciardi, 2005);
- depth (Jones & Ricciardi, 2005);
- transparency (Whittier et al., 1995, Hayward & Estevez, 1997);

- salinity (Strayer & Smith, 1993, Whittier et al., 1995, Hayward & Estevez, 1997, Kozłowski et al., 2002).

To select the suitability criteria which best correspond to the goal of our study and reflect the characteristics of the region, we made a preliminary review of 8 parameters: size of the water body, depth, substrate type, Secchi disk transparency, water temperature, pH, calcium concentration and dissolved oxygen. Since the water bodies in the study region are fresh water, salinity was excluded from the review.

4.2.1 Surface area

The study reservoirs were selected with a surface area over 20 ha. Only 3 of them had smaller surface area (Table 1). The results showed that the biggest in size reservoirs in the study region – Ogosta and Rabisha (with a maximum surface area of 2360 and 324.6 ha, respectively) suffered heavy infestation by zebra mussel. Zebra mussel larvae and shells were found in three of the reservoirs with a surface area between 90 and 120 ha – Drenovets, Kovachitsa, Asparuhov Val. In reservoirs smaller than about 90 ha in surface area, no traces of zebra mussel occurrence were found. The smallest known invaded North American lake was 15 ha (Kraft & Johnson 2000). Reviewing data from 73 European lakes, Strayer (1991) found that zebra mussels rarely invaded lakes smaller than 30 ha. He suggested that zebra mussel absence from shallow, productive lakes could be due either to periods of anoxia, or to intense predation by water birds. Stanczykowska & Lewandowski (1993) similarly found that relatively large and deep European lakes with low to moderate levels of algae and nutrients have higher densities of mussels than relatively small and shallow lakes that are higher in algae and nutrients. The results of the PCA in the study reservoirs showed that 46.2% of total variance of data was explained by the first principal component related to pH, calcium, bicarbonates and electroconductivity (with their principal component loadings -0.814, -0.761, -0.839 and -0.878, respectively). More alkaline and rich in nutrients reservoirs, among them Asparuhov Val and Kovachitsa with traces of zebra mussel infestation, were separated from the lower in nutrients reservoirs, among them Ogosta and Rabisha with heavy zebra mussel infestation (Fig. 8).

Another factors related to the size of the water body are the sources and pathways of zebra mussel introduction. It is assumed that larger water bodies are colonized more easily, presumably due to a greater number of access points and a larger number of human users (Kraft & Johnson 2000, Frischer et al. 2005). Our results confirmed this assumption. The zebra mussel abundance showed a statistically significant positive correlation with number of stockings in the reservoirs (0.73, $P < 0.05$) (Fig. 8). According to the official information about fish stockings in the reservoirs for the period 2001-2005, the highest number of stockings was made in the Rabisha, the reservoir which ranks second in size in the region and which supports the highest abundance of zebra mussel. The reservoir was stocked 12 times. It was followed by the other infested and biggest in size reservoir – Ogosta, as well as the Reservoir Drenovets, where zebra mussel shells were found, each stocked 8 times for this period. The reservoirs Poletkovtsi, Lipnitsa and Kovachitsa were stocked from 4 to 6 times. Four of the reservoirs were stocked less than 3 times and five of the reservoirs were not stocked at all in this period. In the reservoirs without stockings no zebra mussels were found. The exception was Reservoir Asparuhov Val, where a large amount of zebra mussel shells was observed in the littoral near the dam and on the whole shore.

The larger water bodies sometimes offer a higher substrate diversity which may additionally contribute to the more easily colonization of zebra mussel. Such

example is the Ogosta Reservoir, which was constructed by damming the Ogosta River and flooding abandoned settlements, agricultural land, meadows, fruit orchards and vineyards. As a result, its bottom substrate is mixed - from mud and clay to stones and concrete. The hard substrate was preferably colonized by zebra mussels, especially in the early stages of invasion (Stanczykowska 1977, Lewandowski 1982). Moreover, the water bodies with larger surface areas should maintain more constant populations (Ramcharan et al., 1992). In larger water bodies with many diverse habitats, areas with reductions or extinctions of zebra mussels may be repopulated from elsewhere around the water body.

4.2.2. Depth

The results of the PCA in five of the study reservoirs showed that the first principal component related to zebra mussel abundance, Secchi disk transparency, calcium and bicarbonate concentrations and depths of sampling (with principal component loadings 0.941, 0.949, -0.948, -0.935 and -0.820, respectively) explained 51.1% of total variance of data (Fig. 7). Live zebra mussels were not found at sampling sites with depth over 11 m. When all 15 reservoirs were included in the analysis, the importance of sampling depth for explanation of variance of data decreased (its principal component loading was 0.482) (Fig. 8). Live zebra mussels were found at sites with depth of 7.5-8.0 m (Ogosta and Rabisha), while shells of zebra mussels – at 4.5 m (Asparuhov Val), 6.5 m (Kovachitsa) and 12.5 m (Drenovets) (Tables 3).

We studied also the distribution of zebra mussels in the infested reservoirs Ogosta and Rabisha in relation to depth. In these reservoirs, zebra mussels were found at depths between 3.5 and 10 m. Live specimens were not recorded at depths over 13 m. In the Rabisha Reservoir four sites with depths between 13 and 16.5 m were sampled. No live zebra mussels but only shells were found at these sites. According to Stanczykowska (1977) the abundance of zebra mussel populations is the highest in the littoral and sublittoral zones between 2 and 12 m. In the Black Sea coastal lakes Shabla and Ezerets, zebra mussels were found at depths 0.3-8.0 m being most abundant at about 3.5-4.5 m (Kaneva-Abadjieva & Marinov 1967, Valkanov et al. 1978, Liutskanov 1981, 1983, Petrova & Stoykov 2002). In the Danube, zebra mussels occurred at depths between 0.2 and 15.0 m and had highest biomass in the range of 4-7 m (Russev 1966, 1967, 1978). We recorded the highest abundance and biomass at depth of 7.5-8.5 m in the Rabisha Reservoir and 8-9 m in the Ogosta Reservoir. In the shallowest parts of the littoral zones only shells of dead zebra mussels were found. This was probably due to the fact that in the period of sampling the water level in the reservoirs was high. In summer, the level decreases and these parts usually become dry. Similar pattern of distribution with depth, which reflected the zebra mussel tolerance to natural disturbance and periodic aerial exposure was reported by other authors as well (Mitchell et al., 1996, Jones & Ricciardi, 2005). This may be one of the reasons that evidences of zebra mussel occurrence were found only in reservoirs with bigger depths. According to information about depth at construction, two of the study reservoirs have maximum depth over 50 m, eight reservoirs have maximum depth between 20 and 36 m, and five reservoirs – below 15 m (Table 1). Live zebra mussels and shells were recorded in the reservoirs with depths over 22 m. The exception was the Asparuhov Val Reservoir where the maximum depth measured was 4.5 m.

4.2.3. Substrate type

The zebra mussel larvae need hard substrate to settle on and begin adult life. Some authors reported that in the early stages of invasion hard substrate was more important for the settlement and survival of zebra mussel (Stanczykowska 1977, Lewandowski 1982). In the early stages of invasion of the Sr. Lawrence River, the highest zebra mussel densities occurred on hard substrates and substrate size explained 38% of the variation in mussel density (Mellina & Rasmussen 1994). In lakes with little hard substrate, zebra mussels can settle on sticks, logs, or sometimes attach directly to sand grains, and later settle onto each other, eventually forming large mats (Ramcharan et al., 1992, Cohen, 2005). Native unionids and macrophytes are also common substrates for zebra mussel colonization (Lewandowski, 1976, Ricciardi et al., 1996, Diggins et al., 2004). Over time, adult zebra mussels also started to use soft sediments (Haltuch et al. 2000, Jones & Ricciardi 2005), but again preferably colonized hard substrate and their density was determined by substrate size (Dermott & Munawar 1993, Mellina & Rasmussen 1994, Karatayev et al. 1998, Jones & Ricciardi 2005). In the St. Lawrence River, 10 years after invasion, substrate size accounted for 20% of the variation in zebra mussel biomass (Jones & Ricciardi 2005).

The two infested reservoirs Ogosta and Rabisha had mixed type of substrate. The Ogosta Reservoir, which had been constructed by damming the Ogosta River and flooding abandoned settlements, agricultural land, meadows, fruit orchards and vineyards, was characterized by substrate ranging from mud and clay to stones and concrete. In the Rabisha Reservoir - a former tectonic lake, the bottom was sand, gravel, stones and mud, overgrown with submerged macrophytes, such as *Typha* sp., *Potamogeton* cf. *denstum*, etc., in the littoral zone. Our results showed that zebra mussels attached preferably to hard substrate - mainly stones (Ogosta, Rabisha), as well as to tree branches (Ogosta), stems of *Potamogeton* cf. *denstum* (Rabisha), and shells of *Viviparus viviparus* (Rabisha). The zebra mussels were not found on purely clay and muddy substrate. The Kovachitsa Reservoir also had mixed substrate – mud, clay, sand, stones and emergent macrophytes. In this reservoir, large number of shells was recorded in the littoral zone near the dam. Live adult specimens were found only at the shore and most of them were attached to the shells of *Unio pictorum* and *U. tumidus*.

Most of the other reservoirs had also mixed type of substrate, dominated by gravel and stones (Mishkovets), sand and clay (Srechenska Bara, Bozhuritsa), or clay and mud (Christo Smirnenki, Drenovets, Oshane, Poletkovtsi, Kula, Skomlya, Asparuhov Val). The Reservoirs Dabnik and Lipnitsa were characterized by muddy bottom.

4.2.4. Secchi Disk Transparency

As efficient filter feeders, zebra mussels are responsible for the considerable increase in Secchi disk transparency in the infested water basins (Fahnenstiel et al. 1995, MacIsaac 1996). They are capable of removing up to 63% of littoral primary production (Hamburger et al., 1990), up to 5-18% of total lake production (Stanczykowska, 1975), and large amounts of seston from the water (Kryger & Riisgard, 1988). In western Lake Erie, for instance, mean Secchi disk transparency for the May-November period almost doubled between 1988 and 1989 as a result of zebra mussel filtration (Leach, 1993).

The results of the two PCA (Figs 3 and 4) showed that loadings on the first principal component of the Secchi disk transparency were 0.949 and 0.815. In addition, the zebra mussel abundance showed a statistically significant strong positive correlation with transparency (1.00, $P < 0.05$ for the 5 of the reservoirs; 0.74, $P < 0.05$

for the 15 reservoirs). The highest Secchi disk transparency was measured in the infested Reservoirs Rabisha and Ogosta, with maximum value of 370 cm reached in April in the Rabisha (Table 3). Comparatively high Secchi disk value was recorded also in the Srechenska Bara Reservoir. This reservoir is filled with mountain and spring water and used for drinking water supply. Because of the limited activities permitted there, the reservoir has never been stocked and the access of users has been restricted. One of the lowest values of Secchi disk transparency was recorded in the Skomlya Reservoir (Table 3). Because of some reconstructions, its water volume was reduced almost to the dead storage volume. As a result high organic production was observed in the reservoir. Two of the richest in nutrients reservoirs (Lipnitsa and Asparuhov Val, Table 3) were characterized with very low values of Secchi disk transparency as well. However, in the Kovachitsa Reservoir, which was also very rich in nutrients, and where veliger larvae and live zebra mussels at the shore were found, the value of transparency was much higher. It was even higher than the transparency in some reservoirs with moderate nutrient content (Table 3).

In a discriminant function analysis of 30 lakes with and without zebra mussels, Strayer (1991) determined that hardness and lake depth (primarily), and lake area and transparency (to a lesser extent) accounted for 52% of the variation ($F=14.67$, $P<0.01$). He found that zebra mussels were uncommon in lakes with Secchi disk depths under 1 meter. Other authors suggested that high transparency may control zebra mussel distributions by interfering with feeding (Strayer & Smith, 1993). According to some authors, however, transparency was problematic to be used as criteria, because it was difficult to propose tolerance thresholds (Cohen & Weinstein, 1998a). For example, the zebra mussels were found in parts of the Mississippi River, where transparency considerably exceeded levels previously thought to be their upper tolerance limit (McMahon 1996).

4.2.5. Water temperature

Zebra mussels are typically considered a cool water species. Various studies in Europe and North America have reported low temperature limits for adult growth that are in the range of 10-12°C (Stanczykowska 1977, Mackie 1991). In Europe, zebra mussels have become abundant where average winter temperatures are as low as 6°C, but are less common in colder environments (Stanczykowska & Lewandowski 1993). The lower limit for survival of zebra mussels was accepted to be 0°C (McMahon 1996).

Zebra mussel normally begin to spawn at 12°C and above (Neumann et al., 1993), but limited spawning was reported at 10°C (Sprung, 1993, McMahon 1996, Nichols 1996). Spawning peaks at about 12-18°C, which is also roughly the optimum temperature for larval development (Sprung 1993). Juveniles and adults are able to grow at a wide range of temperatures, from about 12°C to about 30°C (Cohen 2005). Several authors reported 30°C as the upper limit for efficient feeding and adult growth, and 31-33°C as the upper limit for short-term survival (McMahon 1996, Cohen 2005). In analysis of potential distribution and abundance of zebra mussel in California, Cohen & Weinstein (1998a) used the following suitability temperature ranges: 15-31°C and $10 \leq \max \leq 31^\circ\text{C}$ for high potential, 0-15°C and $10 \leq \max \leq 31^\circ\text{C}$ for moderate potential, and $\max < 10$ or $> 31^\circ\text{C}$ for low-to-no potential distribution.

The PCA in the study reservoirs showed that the water temperature did not play an important role in explaining total variance of data (loadings on the first and the second principal component 0.334, -549 for the five reservoirs and -0.390, -0.464 for the fifteen reservoirs) (Figs. 3 and 4). At different sampling sites, its values

varied between 8.5 and 13.5°C in April and between 19.6 and 25.1 °C in August-September (Table 3). Maximum water temperature reported for the Rabisha Reservoir in the past was 32°C (Valkanov, 1938). The reservoir freezes regularly in winter. In the Ogosta Reservoir, mean monthly temperatures measured for the period 1998-2006 were 6°C for February and 24°C for August (Bulgarian Ministry of Environment and Water, BMEW). In the Danube and the Black Sea coastal lakes, zebra mussel was reported to occur at temperature range of 0-28°C (Russev, 1966, 1967, 1978, Liutskanov, 1981, 1983).

We analyzed distribution of the water temperature data from April-September (active reproductive months of zebra mussel, Cohen & Weinstein, 1998a) for the Bulgarian part of the Danube River and Black Sea coastal Lake System Shabla-Ezerets, where zebra mussel occurred naturally, as well as for the infested Ogosta Reservoir. Data from the Danube were collected from 24 stations and covered the period 1990-2005 (BMEW). The water temperature varied within a wide range - between 0.4 and 30.6°C with 50% of values being between 8.0°C and 22.2°C, and 25% below 8°C (Fig. 9). The data from the Shabla-Ezerets Lake System covered a smaller period (1992-1994, Botev, 1998) and values varied within a smaller range – between 9.5 and 24.0°C, with 50% of them between 14.2°C and 20.15°C (Fig. 9). Data from the Ogosta Reservoir covered the period 1998-2006 (BMEW), and included our sampling data as well. Temperature ranged from 8.6 to 27.8°C with 50% of values between 16.9°C and 23.25°C, and 25% below 16.9°C (Fig. 9).

Based on the results of PCA, the wide temperature ranges for April-September period in the water bodies where zebra mussel occur, and climatic characteristics of the study region (Koprlev et al., 2002), temperature was not considered as a limiting factor and was not used in the GIS analysis.

4.2.6. pH

pH regulates calcium uptake in freshwater shellfish (Kozłowski et al., 2002). Zebra mussels are generally more vulnerable than other freshwater bivalves to disruption of ion metabolism from reductions in pH level (Vinogradov et al. 1993). They have distinct pH-tolerance limits (Cohen & Weinstein, 1998a). In the laboratory studies, a pH of 7.4 to 9.4 was required for veliger development, and development success peaked at around pH of 8.4 in 18-20°C (Sprung 1993). Ramcharan et al. (1992) analyzed 76 European lakes and found that zebra mussels were absent from those with pH below 7.3. Different authors reviewing the literature have selected minimum pH requirements ranging from 6.5 to 7.5 and maximum pH requirements ranging from 9.0 to 9.5 (Cohen, 2005).

The PCA in five of the reservoirs in the study region showed that 25.9% of variation of data was explained by the second principal component related to the surface area - pH gradient (Fig. 7), with loading of pH on this principal component of -0.916. It separated the more alkaline reservoirs Rabisha, Drenovets and Kula with pH>8 from the reservoirs Ogosta and Poletkovtsi with pH<8. The PCA in all fifteen reservoirs showed that already 46.2% of total variance of data was explained by the first principal component related to pH, calcium and bicarbonate concentrations as well as electroconductivity (loadings on the first principal component -0.814, -0.761, -0.839 and -0.878, respectively) (Fig. 8). More alkaline and richer in nutrients reservoirs – Lipnitsa, Asparuhov Val, Kovachitsa were contrasted to the reservoirs with lower values of pH, calcium, bicarbonates and electroconductivity – Rabisha, Ogosta, Srechenska Bara.

The values of pH measured at different reservoir sampling sites ranged between 7.1 and 8.9 in April, and between 7.8 and 8.8 in August-September. According to published sources, in the Danube and the Black Sea coastal lakes, zebra mussel was found within the range of 7.0-8.9 (Russev, 1966, 1967, 1978, Liutzkanov, 1981, 1983). We analyzed distribution of pH data from April-September period for the Danube River, Black Sea coastal Lake System Shabla-Ezerets, and the infested Ogosta Reservoir. The pH values from the Danube (1954-1961, Rozhdestvenski, 1962; 1990-2005, BMEW) ranged between 6.2 and 9.04 with 50% of values between 7.6 and 8.1 and 25% below 7.6 (Fig. 10). pH from the Shabla-Ezerets Lake System (1992-1994, Botev, 1998) ranged between 7.3 and 8.6, with 50% of values between 7.8 and 8.4 (Fig. 10). Data from the Ogosta Reservoir (1998-2006, BMEW; our data) were in the range of 7.1-9.05 with 50% of values between 7.9 and 8.34 and 25% below 7.9 (Fig. 10).

4.2.7. Calcium concentration

Zebra mussels require calcium for shell growth and osmoregulation (Vinogradov et al. 1993, McMahon 1996). This element was recognized as a key factor affecting the mussels' potential distribution, survival, growth and reproduction (Cohen & Weinstein 2001). Different calcium thresholds were reported in literature. Reviewing data for 70 European lakes, Strayer (1991) found zebra mussels mainly reported in lakes with calcium levels above 20-40 mg/l, and absent from lakes with <20 mg/l. Ramcharan et al. (1992) analyzed 76 European lakes and found that zebra mussels are present only where calcium concentrations are at least 28.3 mg/l. In 527 lakes in Belarus, zebra mussels were found only in lakes with more than 25.4 mg/l of calcium (Karatayev, 1995). In North America, however, zebra mussels have been reported as present and sometimes abundant at calcium levels ranging from 12 to 25 mg/l (Cohen & Weinstein 2001). Laboratory studies found the minimum calcium threshold for normal development and long-term survival of zebra mussels to range from 8.5 to 12 mg/l (Sprung, 1987, Vinogradov et al., 1993, Hincks & Mackie, 1997). Based on field studies, some authors reported no zebra mussels below 15 mg/l (Mellina & Rasmussen 1994, Strayer et al., 1996), and others suggested a threshold for occurrence between 8 and 10 mg/l (Jones & Ricciardi, 2005). After a detailed review of literature, Cohen & Weinstein (2001) summarized that ambient calcium concentration of 12 or 15 mg/l was the minimum threshold below which the establishment of a zebra mussel population was unlikely, and that abundant reproducing populations most probably would become established in concentrations between 20-28 mg/l and above.

Not much was published about upper calcium thresholds. Jones & Ricciardi (2005) found that zebra mussel biomass in the St. Lawrence River increased linearly with increasing calcium concentrations below 15 mg/l, peaked between 23 and 25 mg/l and decreased thereafter. Negative effect of high calcium levels was observed by Hincks & Mackie (1997), who found that adult mortality increased above 25 mg/l and maximum juvenile growth rates decreased above 32 mg/l. In laboratory tests, Sprung (1987) found that when exposed to different calcium concentrations, rearing success and larval condition of zebra mussel were roughly constant for concentrations from about 35 to 106 mg/l.

The results of the PCA in five of the study reservoirs showed that the first principal component related to calcium, bicarbonate concentrations, zebra mussel abundance, Secchi disk transparency and depths of sampling explained 51.1% of total variance of data (loadings on the first principal component for calcium and

bicarbonate concentrations -0.948 and -0.935, respectively) (Fig. 7). Zebra mussel abundance showed a statistically significant strong negative correlation with calcium concentration (-0.91, $P < 0.05$). When all fifteen reservoirs were analyzed the first principal component related to calcium, bicarbonate concentrations, pH and electroconductivity explained 46.2% of total variance of data (loadings on the first principal component for calcium and bicarbonates -0.761 and -0.839, respectively) (Fig. 8). In April, the lowest values of calcium concentration were measured in the infested reservoirs Ogosta and Rabisha (Table 3). There were almost no differences in calcium concentration between different sampling sites and profiles within a reservoir. In August-September, the calcium concentration varied within a wide range among different reservoirs (Table 3). The lowest value (8.04 mg/l) was measured in the reservoir filled mainly with mountain water - Srechenska Bara. The Reservoirs Ogosta and Rabisha again had some of the lowest values measured – 22.04 and 20.04 mg/l, respectively. In other reservoirs with moderate nutrient content, calcium concentration was in the range from 20.04 to 50.10 mg/l. In the reservoirs with high nutrient content, calcium concentration was between 82.16 and 148.3 mg/l (Table 3). It was the highest in the Asparuhov Val Reservoir, where large amounts of zebra mussel shells existed, and the lowest in the Kovachitsa Reservoir, where zebra mussel veliger larvae were found. The strong negative correlation of calcium concentration and zebra mussel abundance may reflect more the effect of the zebra mussels than their requirements. The results suggested that in the infested reservoirs calcium was reduced because of intensive use by zebra mussel for shell production and growth. On the other hand, the calcium measured in infested water basins reflect the concentrations required to support existing populations and they may be different from these needed for zebra mussel to become established (Murray et al., 1993).

The result of analysis of data on calcium concentration from April-September period for the Danube River and the Black Sea coastal Lake System Shabla-Ezerets, where zebra mussels occur naturally, and the infested Ogosta Reservoir are shown on Fig. 11. The values of calcium concentration from the Danube (1954-1961, Rozhdestvenski, 1961; 1990-2005, BMEW) ranged between 12.20 and 120.00 mg/l with 50% of values between 44.0 mg/l and 57.1 mg/l and 25% below 44.00 mg/l. Calcium concentration from the Shabla-Ezerets Lake System (1992-1994, Botev, 1998) ranged between 26.00 and 50.10, with 50% of values between 40.1 mg/l and 44.1 mg/l (Fig. 11). Data from the Ogosta Reservoir (2005-2006, BMEW; our data) were in the range of 22.00-38.10 mg/l with 50% of values between 24.05 mg/l and 32.07 mg/l and 25% below 24.05 mg/l (Fig. 11). It is illustrated that calcium concentration in the infested Ogosta Reservoir varied in a lower range and had lower values than the other two water bodies where zebra mussel occur naturally.

4.2.8. Dissolved oxygen

Zebra mussels are among the least tolerant of low oxygen levels of all freshwater bivalves (Cohen & Weinstein, 1998a). The lethal lower limit for adult zebra mussels is about 4 mg/l of oxygen, or about 20% of saturation at 18°C (Sprung, 1987). Some authors reported that zebra mussels were usually found where dissolved oxygen was over 90% of saturation and become stressed at levels of 40-50% of saturation; others reported 80-85% oxygen saturation as optimal (Cohen, 2005). The oxygen requirements of zebra mussel rose in warm water (25°C and over), and decline in colder water allowing overwintering mussels to survive under ice (Cohen & Weinstein, 1998a). Low oxygen levels may in part account for the poor colonization success of zebra mussels in eutrophic lakes (McMahon, 1996).

Dissolved oxygen concentration at different sampling sites in the study reservoirs ranged from 7.8 to 12.0 mg/l (93-110% oxygen saturation) in April, and from 6.5 to 17.0 mg/l (72-215%) in August-September (Table 3). In the late summer, the infested reservoirs Ogosta and Rabisha were among the reservoirs with the lowest oxygen concentrations measured. The Reservoir Kovachitsa had the lowest dissolved oxygen compared to the other two rich in nutrients reservoirs (Lipnitsa and Asparuhov Val). According to literature, in other Bulgarian water basins, where zebra mussels occurred, oxygen content detected was between 2.35 and 10.17 mg/l (Liutzkanov 1981, 1983). In the Danube, the oxygen concentration ranged from 6.58 to 8.83 mg/l and oxygen saturation - from 80 to 97% (Russev 1966, 1967, 1978). We analyzed data on dissolved oxygen from April-September period for the Danube River, Black Sea coastal Lake System Shabla-Ezerets, and the infested Ogosta Reservoir. The dissolved oxygen concentration in the Danube (1990-2005, BMEW) ranged between 2.85 and 14.60 mg/l with 50% of values between 6.80 mg/l and 9.23 mg/l and 25% below 6.80 mg/l (Fig. 12). The oxygen concentration in the Shabla-Ezerets Lake System (1992-1994, Botev, 1998) ranged between 0.16 and 11.20 mg/l, with 50% of values between 4.80 mg/l and 7.92 mg/l and 25% below 4.80 mg/l (Fig. 12). Data from the Ogosta Reservoir (1998-2006, BMEW; our data) were in the range of 5.43-17.70 mg/l with 50% of values between 6.90 mg/l and 9.60 mg/l and 25% below 6.90 mg/l (Fig. 12).

4.3. Habitat suitability criteria

The principal component analysis used showed that calcium concentration, pH, electroconductivity and Secchi disk transparency were most important parameters in explaining the majority of total variance of data. Based on these results, the review of the eight physicochemical parameters related to zebra mussel physiological and habitat requirements, and the available data, we selected calcium concentration, pH and dissolved oxygen as criteria in the GIS assessment. The habitat suitability ranges defined for each of the selected criteria were adapted from Cohen & Weinstein (1998a) (Table 4). Cohen & Weinstein (1998a, b) analyzed the potential distribution and abundance of zebra mussel in California using tolerance ranges based on the environmental requirements of zebra mussels during the larval and early growth stages, which were more restrictive than those of adults. For instance, adult mussels can tolerate pH levels as low as 6.5-7.0 and calcium levels as low as 12-15 mg/l, while larvae need more alkaline conditions, of at least pH 7.3-7.4 and calcium 15 mg/l (McMahon, 1996, Cohen & Weinstein, 1998a). In their analysis, they used average April through September values, the main period for growth and reproduction of zebra mussel. In our analysis, we also used mean values for April-September period. Although oxygen did not play an important role in explaining total variance of data in the PCA, it was selected as a suitability criteria because of the very low tolerance especially of larval zebra mussels to low dissolved oxygen conditions. However, it received a lower weight than calcium concentration and pH:

- Calcium – 40 %
- pH – 40 %
- Dissolved oxygen – 20 %.

4.4. Potential risk of infestation

The study region was first classified according to pH data and a map with three classifications was produced (Fig. 13 and 14). The areas with mean pH values for the period April-September in the range of <7.3 and >9.0 covered 13.02% of the territory. These included mainly the upper reaches of rivers, where pH was below 7.3. Three small areas along the middle reaches of the Ogosta River, down reaches of the Lom River and the Irrigation Canal System around Vidin also had mean values of pH within this range. Probably, these parts of the rivers suffered some local negative impact which resulted in increased eutrophication or pollution. The Reservoir Srechenska Bara used for drinking water supply was included in this area. In August-September, we also measured the lowest pH value in this reservoir (Table 3).

22.61% of the territory was classified with pH values within the ranges of 7.3-7.5 and 8.7-9.0 (Fig. 14). This area covered mainly the upper and middle reaches of rivers. The Reservoirs Kula, Poletkovtsi, Oshane, Skomlya, Dabnik and the infested Rabisha were included in this area. The majority of the territory (64.36%) was classified with pH values in the range of 7.5-8.7 (Fig. 14). It covered mainly middle and down reaches of rivers but also the upper reaches of some rivers. The study Reservoirs Bozhuritsa, Drenovets, Christo Smirnenki, Lipnitsa, Asparuhov Vala and the infested Ogosta and Kovachitsa belonged to this area. Our analysis of distribution of pH data monitored in the Ogosta Reservoir in the period 1998-2006, including our survey data, confirmed these results. The April-September pH values ranged from 7.1 to 9.05 with 50% of values between 7.9 and 8.34 and mean value of 8.08 (Fig. 10). However, when analyzing the water chemical parameters, we must consider the fact that they undergo significant diurnal and seasonal variations in reservoirs. Variations in the littoral zone, where zebra mussel colonization would be most probable, could be significantly greater. pH would fluctuate in the inshore areas with decreases occurring in the spring associated with snow-melt and runoff from adjacent shorelands and increases occurring later in the summer associated with respiration.

Based on an interpolation technique which combined geology (limestone rock formation spatial distribution, Fig. 4), and existing spatial data from monitory stations, a map predicting the calcium distribution in the North-West Bulgarian waters was produced (Fig. 15, 16). The calcium concentration in the water basins within the study region for the period April-September varied in a wide range - from 1.8 to 148.3 mg/l. Its distribution showed a strong correlation with location and extent of limestone rock formations. A tendency of cumulative increase downstream was observed. According to the calcium suitability ranges of larvae zebra mussels (Table 4), three classifications were made (Fig. 17). 27.64% of the territory was classified with calcium concentration below 15 mg/l. This included mainly the upper reaches of rivers. Only Srechenska Bara and Mishkovets from study reservoirs were included in this area. These are the reservoirs with the highest altitude. During our survey, the lowest calcium concentration was measured in the Srechenska Bara Reservoir (Table 3).

A small share of the territory (11.46%) was classified with calcium concentration in the range from 15 to 25 mg/l (Fig. 17). The two infested Reservoirs Rabisha and Ogosta were included in this area. The data about distribution of calcium concentration for April-September in the Ogosta Reservoir did not correspond to these results. It was illustrated that calcium concentration in the reservoir varied in a lower range and had lower values than the Danube River and Shabla-Ezerets Lake System where zebra mussel occur naturally (Fig. 11). However, data for the period 2005-2006, including our data, were in the range of 22.00-38.10 mg/l with 50% of values between 24.05 mg/l and 32.07 mg/l and a mean value of 28.97 mg/l (Fig. 11).

Obviously the calcium concentration in the rivers was lower. In the Rabisha Reservoir, values below 25 mg/l were measured in April as well as in August-September (Table 3). These results may suggest, that larval zebra mussels can tolerate well calcium concentrations below 25 mg/l and that value dividing moderate from high infestation potential can be lower.

The major part of the territory (60.90%) was classified with calcium concentration over 25 mg/l (Fig. 17). It included the middle and down reaches of rivers and the rest of the study reservoirs. The Reservoirs Kovachitsa, Lipnitsa and Asparuhov Val were characterized with the highest calcium concentrations (Fig. 2, Table 3). The Kovachitsa Reservoir was found to be infested by zebra mussel. In the Asparuhov Val Reservoir, a large number of zebra mussel shells covered the littoral zone near the dam as well as the entire shore. However, no live specimens and veliger larvae in the plankton were found. The reasons for the massive die-off of zebra mussels were not clear. Possible explanation may be the increased eutrophication.

As a result of estimation based on catchment elevation as a proxy of atmospheric pressure and average slope, as well as spatial data from monitory stations, a map predicting the distribution of dissolved oxygen in the North-West Bulgarian waters was produced (Fig. 18). The mean dissolved oxygen concentrations for the period April-September ranged from 6.91 to 11.91 mg/l. According to the dissolved oxygen suitability ranges of larvae zebra mussels (Table 4), two classifications of the territory were made. 42,71% of the territory was classified with oxygen concentration in the range from 6 to 8 mg/l (Fig. 19). It covered mainly the upper and middle reaches of rivers. The Reservoirs Kula, Poletkovtsi, Skomlya, Christo Smirnenki, Srechenska Bara and Mishkovets were included in this area. The other 57,29% of the territory was classified with oxygen concentration over 8 mg/l (Fig. 19). It covered middle and down reaches of rivers. All the infested reservoirs were included in this area. The distribution analysis of dissolved oxygen concentration in the Ogosta Reservoir for the period 1998-2006, including the survey data, showed that, the values were in the range of 5.43-17.70 mg/l with 50% of them between 6.90 mg/l and 9.60 mg/l and mean value of 8.45 mg/l (Fig. 12).

The oxygen concentrations like pH also undergo significant diurnal and seasonal variations. They are usually higher in winter than in summer. During dry seasons, water levels decrease and the dissolved oxygen concentrations also decrease, while during rainy seasons, they tend to be higher. The Reservoirs Kovachitsa, Lipnitsa and Asparuhov Val were characterized with high nutrient content and some of the highest oxygen concentrations and oxygen saturation over 100% were recorded there (Table 3). As a result of intensive photosynthesis of algae and macrophytes, supersaturation with dissolved oxygen occurred during the day. Most probably, concentrations of oxygen decrease significantly during the night, due to respiration. Dissolved oxygen concentrations are usually highest in the late afternoon, because of the all day photosynthesis. These variations, especially the decline of oxygen saturation during the night, can be stressful for zebra mussels. Such conditions were observed in the Rabisha Reservoir, whose littoral zone was densely overgrown with submerged macrophytes.

Data about pH, calcium and dissolved oxygen concentrations of varying suitability for zebra mussel larvae survival in the study region were integrated with the help of weighted overlay technique. A map with the distribution of these parameters which indicated the infestation potential of zebra mussel was produced (Fig. 20). 20.74% of the territory of the region was classified with low potential, 15.45% - with moderate potential and 63.81% - with high potential for zebra mussel

infestation (Table 5). The areas with low and moderate potential cover mainly the upper and part of middle reaches of rivers. The area with high potential of zebra mussel infestation cover the middle and down reaches of rivers. The majority of the reservoirs in the region are included in this area.

4.5. Potential sources of introduction

The study water bodies are located within the catchment and in the proximity to the Danube River, which is the native range of distribution of zebra mussel. The upstream and overland dispersal of zebra mussel in most of the cases is associated with human activities (Johnson & Carlton 1996, Johnson & Padilla 1996, Johnson et al., 2001, Minchin et al. 2003, Frischer et al. 2005). So, to evaluate the zebra mussel dispersal mechanisms in the region, we considered the following factors:

- Proximity of the reservoirs to the Danube or already infested water bodies
- Accessibility of the reservoirs by human users
- Stocking with juvenile fishes hatched in fish-farms located along the Danube
- Transport and use of fishing equipment (nets, boats) from the Danube.

The main use of the reservoirs in the past was for irrigation. To ensure constant water supply in summer months many of the reservoirs were connected through artificial derivative channels to bigger rivers or other reservoirs (Table 1). This is most probably the main reason for the infestation of the reservoirs located closest to the Danube (Kovachitsa and Asparuhov Val). The Reservoirs Kovachitsa and Asparuhov Val were connected with the Danube through artificial channels and in the past they were filled with water from the Danube. At present the reservoirs are not used for irrigation and the pumping stations are not working. The Asparuhov Val Reservoir has not been filled with Danube water since 1993. The large amount of zebra mussel shells in the reservoir and along the shores was an evidence of heavy infestation of this reservoir in the past. One possible reason for the massive die-off of zebra mussels could be the increased eutrophication in the reservoir (Table 3). In the past, the high population density was sustained by constant and repeated introduction of larvae from the Danube. After the connection with the Danube was interrupted in 1993, the drift of larvae ceased and the population gradually declined. In the Reservoir Kovachitsa, which is less eutrophicated, still a reproducing population has been maintained though in very low abundance. It is difficult to make any conclusions because of the lack of enough data.

The Rabisha Reservoir is connected with the upstream located Oshane Reservoir through a small river and an artificial channel (Table 1). Our survey did not show any evidence of zebra mussel infestation in this reservoir.

It is assumed that larger water bodies are colonized more easily, presumably due to a greater number of access points and a larger number of human users (Kraft & Johnson 2000, Frischer et al. 2005). Our results to some extent confirmed this assumption. The largest in surface area reservoirs in the region Ogosta and Rabisha supported abundant zebra mussel populations. The Reservoir Ogosta is located nearby the town of Montana. It is very easily accessible and frequently visited by local people for recreation, motor boating and sports fishing. The Reservoir Rabisha is situated in the vicinity of the Magurata Cave which is a popular tourist attraction. It is also frequently visited by tourists, local people and fishermen, and known for the angler's competitions organized there. One possible mechanism for the introduction of zebra mussel to these two reservoirs was the transport of larvae or adult individuals with boats and fishing equipment (nets, live bait) from the Danube. Though the number of

boats in the region was not big, according to our observations, it is a common practice for fishermen to use their boats in the Danube and then in the inland reservoirs. The recreational boating has become popular only recently but it also can be identified as a potential mechanism of dispersal. There are number of reports in the literature suggesting that boating, mainly recreational, involving boat movement between unconnected water bodies, is an important overland zebra mussel transport vector (Koutnik & Padilla 1994, Kraft & Johnson 2000, Johnson et al., 2001, Frischer et al., 2005).

Another potential source of zebra mussel introduction to the reservoirs is fish stocking. Since most of the reservoirs have been used for recreational and commercial fishing (Table 1), some of them were regularly stocked. We included data about official fish stocking in our analysis. The results of the PCA showed that the first two principal components, related to calcium, bicarbonate concentrations, pH, electroconductivity, Secchi disk transparency as well as zebra mussel abundance and number of fish stockings, explained cumulatively 64.5% of total variance of data (Fig. 8). The infested reservoirs with higher values of Secchi disk transparency and higher number of fish stockings – Ogosta and Rabisha were separated from the other reservoirs with low value of transparency, without live zebra mussels and lower number of fish stockings. The zebra mussel abundance showed a statistically significant positive correlation with number of stockings (0.73, $P < 0.05$). According to official information for the period 2001-2005, the highest number of fish stockings was made in the Rabisha Reservoir (Table 1). The reservoir was stocked 12 times mainly with carp and bighead carp. It was followed by the Reservoirs Ogosta and Drenovets, each stocked 8 times for this period. The Ogosta Reservoir was stocked mainly with carp and silver carp. The Drenovets Reservoir, where small number of zebra mussel shells was found, was stocked with carp and bighead carp in 2002-2003. In the period 2004-2005 the reservoir was used for aquaculture. Paddlefish was introduced to the reservoir and Russian sturgeon was reared in cages. In 2006, the sturgeon in the cages was replaced by carp. The reservoirs Poletkovtsi, Lipnitsa and Kovachitsa were stocked 4-6 times for this period mainly with cyprinids. The Reservoir Lipnitsa is used for rearing of stocking material. Every year it is stocked with newly hatched cyprinids. In the period 2001-2005, four of the reservoirs were stocked less than 3 times and five of the reservoirs were not stocked at all. In the reservoirs without stockings no zebra mussels were found. The exception was Reservoir Asparuhov Val, which was most probably infested as a result of its direct connection with the Danube.

Most of the carp stocking material for the reservoirs was taken from one fish farm, which is the biggest in the region. The cyprinids were bred and hatched in a small fish pond reservoir and after that transported to the Lipnitsa Reservoir for feeding. Carps were fed mainly with artificial food. Our results showed that the Lipnitsa Reservoir was rich in nutrients and with very low values of Secchi disk transparency. The bottom substrate was purely anaerobic mud. Every year in winter the reservoir was emptied, cleaned, disinfected and in spring again stocked with newly hatched cyprinids. No adult, larvae or shells of zebra mussels were found there during our sampling. The substrate type, water chemistry and regime of utilization of the reservoir do not seem suitable for settlement and establishment of zebra mussels. However, it is possible in some of the years zebra mussel larvae or adults be transported if water from the Danube was used. Moreover, according to some information the reservoir is connected to the infested Kovachitsa Reservoir by the Lipnitsa River (Table 1).

The sturgeon stocking material reared in the Drenovets Reservoir was hatched at a sturgeon fish farm in the town of Vidin. The fish farm is located at the bank of the Danube River and uses water from the Danube. There was a great probability some larvae, adults or shells of zebra mussel be transported with juveniles, water or sand from the fish farm to the reservoir. The fact that we found only small amount of shell pieces without larvae in the plankton suggests that probably no live zebra mussels but only shells were transported.

Despite official fish stocking, the unofficial introduction of different fish species into the reservoirs may be identified as an important potential dispersal mechanism of zebra mussels as well. According to our information, local people, fishermen, and even reservoir owners, release fish species caught in the Danube, such as wels catfish (*Silurus glanis*), bream (*Abramis brama*), pike (*Esox lucius*), etc., into the reservoirs. An evidence of this is the occurrence of some typical Danube species that are not target of official stocking in the reservoirs. For instance, bream was found in the Reservoirs Ogosta and Poletkovtsi, sneep (*Chondrostoma nasus*) and monkey goby (*Neogobius fluviatilis*) - in the Ogosta Reservoir.

5. Conclusions and Recommendations

As a result of our assessment, the following conclusions were made:

- Based on water chemistry and environmental suitability ranges of zebra mussels during the larval and early growth stages, 63.81% of the North-West Bulgarian territory was classified with high potential, 15.45% - with moderate potential, and 20.74% - with low potential for zebra mussel infestation. Thus, the North-West Bulgaria was identified as a region with very high risk of zebra mussel infestation.
- In reservoirs, the large surface area (over 90 ha) and depth, high substrate diversity, moderate amount of nutrients and easy accessibility to human users, were additional factors that contributed to the increased risk of infestation by zebra mussel.
- As potential zebra mussel dispersal mechanisms in the region were identified the direct waterway connections with the Danube, as well as transport of larvae or adult individuals with fishing equipment (nets), boats and fish stocking material from the Danube and fish farms nearby; and from already infested water bodies, such as the Reservoirs Ogosta, Rabisha and Kovachitsa.

To protect this and other vulnerable regions in Bulgaria from further zebra mussel invasions, it is recommended:

1. Interested governmental institutions (Ministry of Environment and Waters, Bulgarian Agency of Fisheries and Aquaculture, Ministry of Education and Science, etc.) should be informed about the problem in order further actions be initiated. These should focus on:

- Public education and awareness of zebra mussels biological, economic and social impact
- Understanding of the problem by local reservoir managers and interested private companies in order to guide sampling efforts and to assist in taking preventive measures
- Performing of regular water chemical and biological monitoring in the region
- Strong control on fishery, fish-farm and fish stocking activities.

2. Continue the research activities by developing a Risk Management Plan which should be linked to the evaluation of potential biological and economic consequences of zebra mussel invasions. This should include:

- Further development of the created GIS database by adding updated and more detailed data layers as data become available in the future
- Development of early warning system based on the continuously updating GIS database
- Development of risk management measures
- Directing efforts to preserve the biodiversity and natural ecosystems from invasions

Thus, a Risk Assessment and Decision Analysis guide to potential invasion sites in other regions of Bulgaria will be provided.

Acknowledgements: The authors would like to thank the Regional Departments of the Bulgarian Agency of Fisheries and Aquaculture in Vidin, Montana and Vratsa and their Chiefs Mr Milen Metodiev, Mr Rumen Tsankov and Mr Petar Ivanov for providing data about reservoirs and for co-operation during field work. The authors thank also the MSc students Nedyalko Kargov, Maria Doleva, Milena Pavlova and Venelin Hubenov for the assistance in laboratory processing of samples.

The authors are especially grateful to Dr. Robert Kennedy for all his valuable advices and support during the preparation and implementation of the project.

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- Hubenov, Z., 2005. *Dreissena* (Bivalvia: Dreissenidae) – systematics, autochthonous and anthropogenic areas. *Acta zoologica bulgarica*, 57 (3): 259-268.
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- Trichkova, T., D. Kozuharov, Z. Hubenov, I. Botev, M. Zivkov, S. Cheshmedjiev. Characteristics of zebra mussel (*Dreissena polymorpha*) populations in infested reservoirs, North-West Bulgaria. Manuscript submitted to Journal of Natural History, London. (under review)
- Trichkova, T., D. Kozuharov, A. Popov, A. Kotsev, Z. Hubenov, I. Botev, M. Zivkov. Assessment of Zebra Mussel (*Dreissena polymorpha*) Infestation Risk Using GIS for Water Basins in the North-West Bulgaria. Abstract submitted to the 15th International Conference on Aquatic Invasive Species (ICAIS), 23-27 September 2007, Nijmegen, Netherlands.

Table 1. Characteristics of the reservoirs sampled in the North-West Bulgaria.

Reservoir	Reservoir code	River Catchment	Latitude N (degrees)	Longitude E (degrees)	Altitude, m a.s.l.	Max surface area, ha	Max storage volume, m ³ x10 ³	Max depth, m	Filled from	Use
Kula	K	Topolovets	43,54582	22,31373	210	156,7	20250	31	Topolovets River	Irrigation, recreational fishing
Poletkovtsi	Pol	Voinishka	43,50570	22,29300	196	164,8	17500	36	Tributaries of Voinishka River	Irrigation, electricity production, commercial and recreational fishing
Bozhuritsa	Bozh	Vidbol	43,53412	22,44410	87	23,7	1430	13	Vidbol River	Irrigation, recreation, recreational fishing
Rabisha	Rab	Archar, Vidbol	43,44169	22,34022	302	324,6	43200	22	Artificial derivations, connected with Oshane Reservoir	Irrigation, recreational fishing
Oshane	Osh	Archar	43,41373	22,33391	347	8,4	609	20	Oshane River	Irrigation, recreational fishing
Skomlya	Sk	Skomlya	43,40265	22,44475	273	11,4		<15	Skomlya River	Irrigation
Christo Smirnenski	ChSm	Lom	43,36564	23,04560	164,6	97,5	9000	25	Nechinska Bara River	Production of electricity, recreational fishing
Drenovets	Dr	Lom	43,41450	22,54556	188	107	12000	28	Medovnitsa River, derivation from Lom River	Irrigation, aquaculture, commercial and recreational fishing
Lipnitsa	Lip	Tsibritsa	43,47223	23,26583	65,6	46,5	955	7,8	Lipnitsa River	Aquaculture
Kovachitsa	Kov	Tsibritsa	43,47379	23,21450	117,5	112	8020	25	Lipnitsa River, Danube River in the past	Irrigation, recreational fishing
Ogosta	Og	Ogosta	43,23357	23,12532	202,5	2360	506000	56	Rivers Ogosta, Barziya and Zlatitsa	Irrigation and prduction of electricity, recreation, recreational fishing
Mishkovets	M	Ogosta	43,15750	23,72100	446	4,4		<15	Tributary of Barziya	Recreational fishing
Srechenska Bara	SrB	Ogosta	43,12408	23,11574	445	75		52	Mountain water (90%) and spring water	Drinking water supply
Dabnik	Dab	Ogosta	43,12486	23,35040	354	80		20-36	Tributary of Varteshnitsa River	Recreational fishing
Asparuhov Val	AsV	Ogosta	43,47324	23,21520	108	98,6	8000	<15	Danube River before	Irrigation, recreational fishing

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Table 2. Period of sampling and number of sampling sites at the reservoirs.

Reservoir	Reservoir Code	Date of sampling	Number of sampling sites		
			Water chemistry	Adult zebra mussels	Veliger larvae
Kula	K	20.04.2006	3	6	6
		05.09.2006	3	4	3
Poletkovtsi	Pol	19.04.2006	3	5	3
		05.09.2006	3	3	3
Bozhuritsa	Bozh	04.09.2006	1	10	1
Rabisha	Rab	18.04.2006	3	7	3
		02.09.2006	3	4	3
Oshane	Osh	02.09.2006	1	8	1
Skomlya	Sk	03.09.2006	1	1	1
Christo Smirnenski	ChSm	31.08.2006	2	3	2
Drenovets	Dr	17.04.2006	3	3	3
		01.09.2006	3	9	3
Lipnitsa	Lip	06.09.2006	1	6	1
Kovachitsa	Kov	06.09.2006	1	10	1
Ogosta	Og	14-16.04.12006	5	15	5
		30.08.2006	5	5	5
Mishkovets	M	29.08.2006	1	1	1
Srechenska Bara	SrB	29.08.2006	2	2	2
Dabnik	Dab	28.08.2006	1	2	1
Asparuhov Val	AsV	07.09.2006	1	12	1

Table 3. Physicochemical parameters, zebra mussel occurrence, abundance and biomass and number of fish stockings in the reservoirs sampled in the North-West Bulgaria.

Reservoir	Depth, m	Water temp, oC	Secchi Disk transparency, cm	Dissolved oxygen, mg/l	Oxygen saturation, %	pH	Electroconductivity, µS/cm	HCO ₃ ⁻ , mg/l	Ca ²⁺ , mg/l	Mean abundance of zebra mussel, ind./m ²	Mean biomass of zebra mussel, g/m ²	Presence of zebra mussel shells	Presence of veliger larvae in the plankton	Number of stockings (2001-2005)
April 2006														
Kula	18	8,5	60	12	110	8	-	173,9	42,08	0	0	-	-	
Poletkovtsi	16	9,3	40	7,8	71	7,8	-	169,3	45,09	0	0	-	-	
Rabisha	8	10,4	370	9,6	93	8,5	-	82,4	23,05	3910	1559,6	Yes	Yes	
Drenovets	11	11,5	75	10	99	8,3	-	180	46,09	0	0	-	-	
Ogosta	7	9,3	114	9,52	95	7,5	-	103,7	32,06	681	379	Yes	Yes	
August-September 2006														
Kula	6,5	24	150	9,07	107,2	8,35	374	143,3	26,05	0	0	-	-	3
Poletkovtsi	9,5	23,5	55	10,01	117	8,65	328	207,4	50,1	0	0	-	-	6
Bozhuritsa	4	21,4	52	14,4	171	8,37	460	216,6	42,08	0	0	-	-	3
Rabisha	7,5	19,7	250	7,2	72	8,02	177,7	85,4	20,04	2312	1302,6		Yes	12
Oshane	7,5	20,4	80	10,1	117	8,4	262	183	40,08	0	0	-	-	0
Skomlya	1	25	25	17	215	8,4	577	256,2	20,04	0	0	-	-	0
Christo Smirnenski	9	20,5	50	8,12	92,8	8,25	275	183	36,07	0	0	-	-	0
Drenovets	12,5	21,4	70	6,53	73	7,97	276	143,3	26,05	0	0	Yes	-	8
Lipnitsa	3,5	22	25	10,4	121,2	8,5	916	594,8	136,27	0	0	-	-	5
Kovachitsa	6,5	22,3	95	9,16	107	8,55	672	475,8	82,16	0	0	Yes	Yes	4
Ogosta	8	22,2	205	6,7	75	8,2	167,8	97,6	22,04	821	789,5	-	Yes	8
Mishkovets	1,8	23,2	70	6,78	83,8	8,2	180	97,6	22,04	0	0	-	-	2
Srechenska Bara	8	25,1	190	8,4	107	7,9	63,8	42,7	8,02	0	0	-	-	0

Dabnik	15	22,7	70	7,77	96	8,2	397	134,2	37,08	0	0	-	-	1
Asparuhov Val	4,5	24,6	35	10,5	126,8	8,72	886	512,4	148,3	0	0	Yes	-	0

Table 4. Suitability ranges for the physicochemical parameters used in the analysis (according to Cohen & Weinstein, 1998a).

	High Potential	Moderate Potential	Low Potential
pH	7.5-8.7	7.3-7.5 or 8.7-9.0	< 7.3 or > 9.0
Calcium concentration (mg/l)	> 25	15-25	< 15
Dissolved oxygen (mg/l)	> 8	6-8	< 6

Table 5. Infestation potential of zebra mussel in the North-West Bulgaria.

Infestation Potential	Area (km ²)	Relative Share (%)
Low	1888.11	20.74
Moderate	1407.09	15.45
High	5810.18	63.81



Fig. 1. Map of the North-West Bulgaria with the main watersheds.

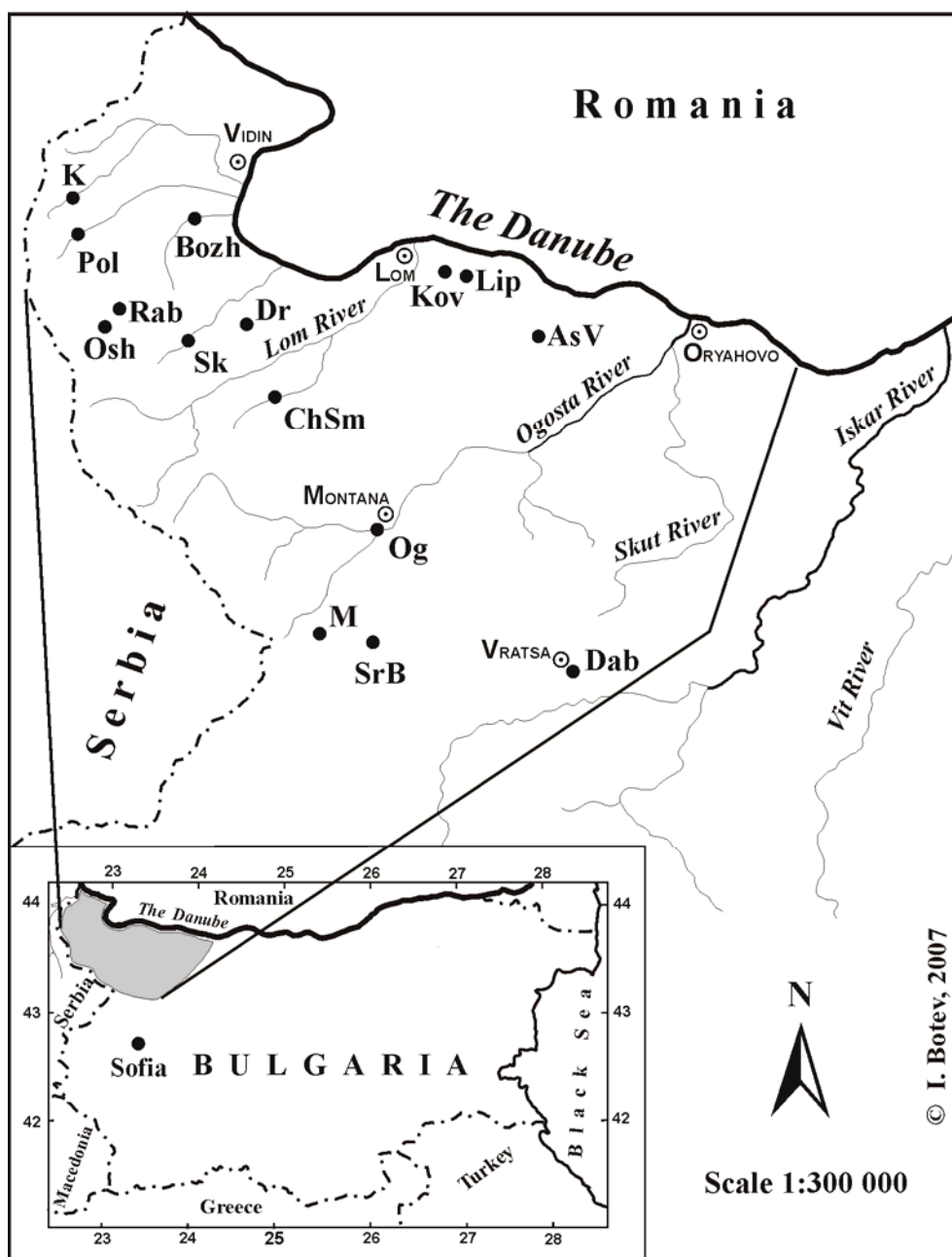


Fig. 2. Study region and survey reservoirs. For reservoir codes see Table 1.

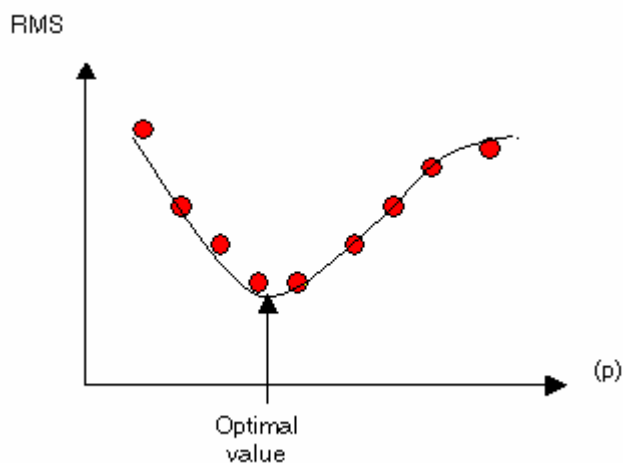


Fig. 3. Optimal prediction values relative to the Root Mean Square (RMS) error; ArcGIS Geostatistical Analyst 9.2.

Relative Share of the Territory of Catchments in NW Bulgaria Covered by Limestone

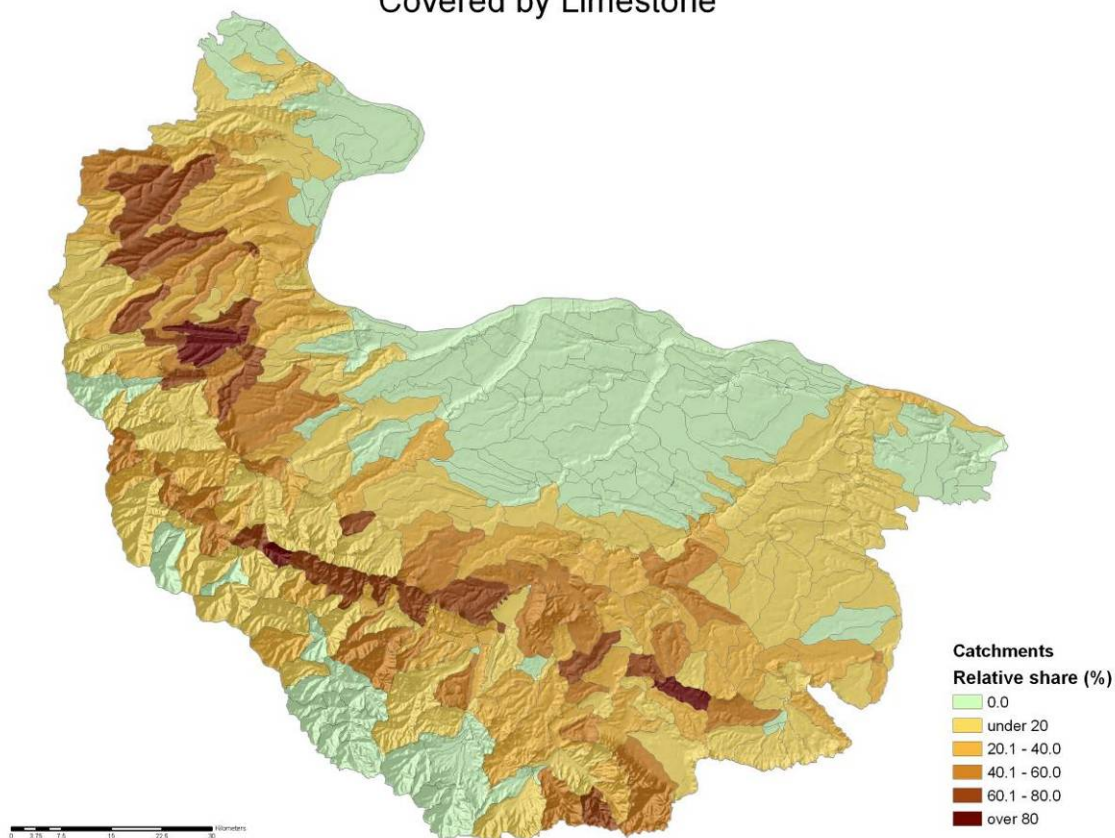


Fig. 4. Relative share of the territory of catchments covered by limestone in the North-West Bulgaria.

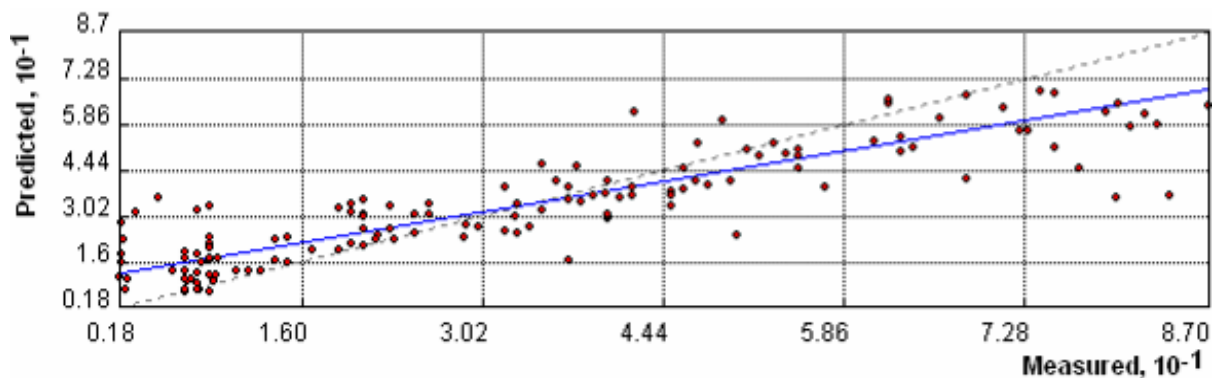


Fig. 5. Correlation of predicted and measured values of calcium; ArcGIS Geostatistical Analyst 9.2.

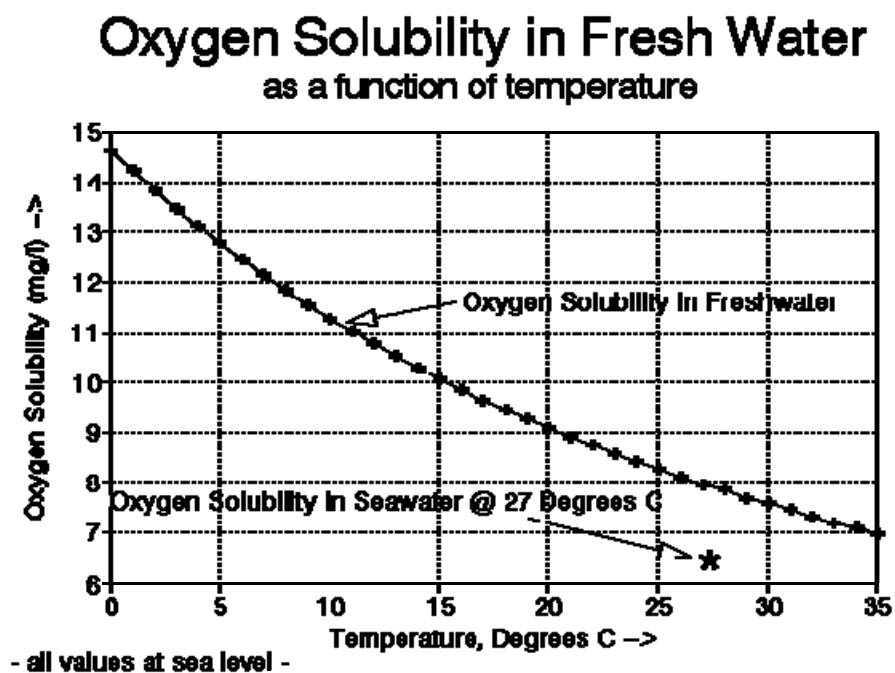


Fig. 6. The oxygen saturation as a function of temperature at a total pressure of 760 torr in a water-vapour saturated atmosphere according to Truesdale, Downing & Lowden, J. Appl. Chem. 5, 53 (1955).

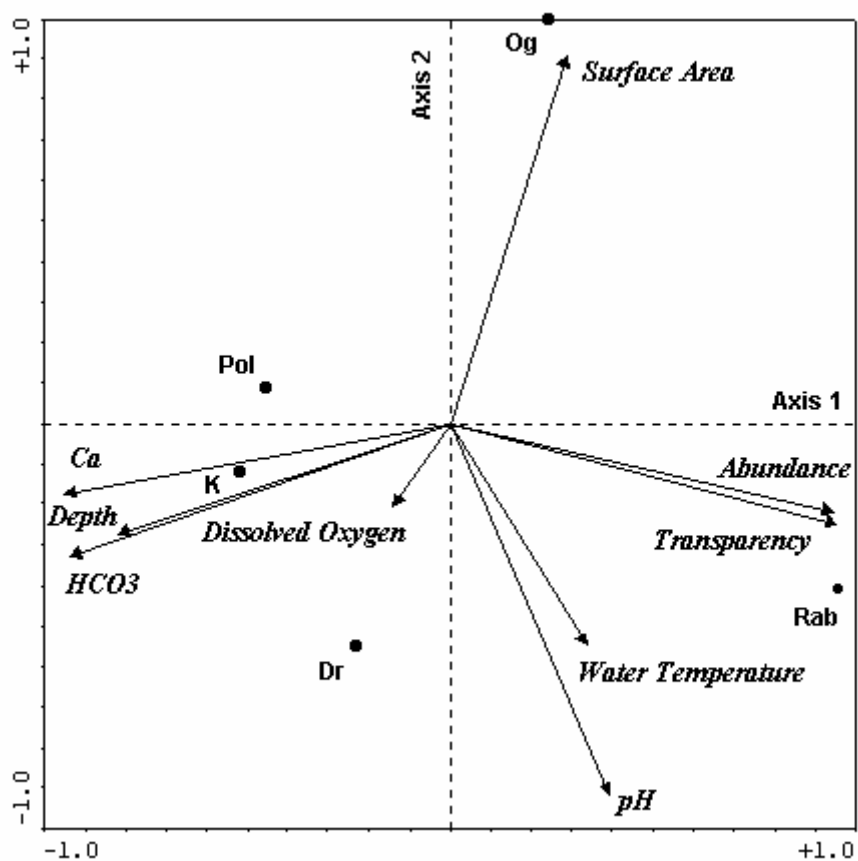


Fig. 7. Principal component analysis (PCA) correlation biplot of the data obtained in April 2006. For reservoir codes see Table 1.

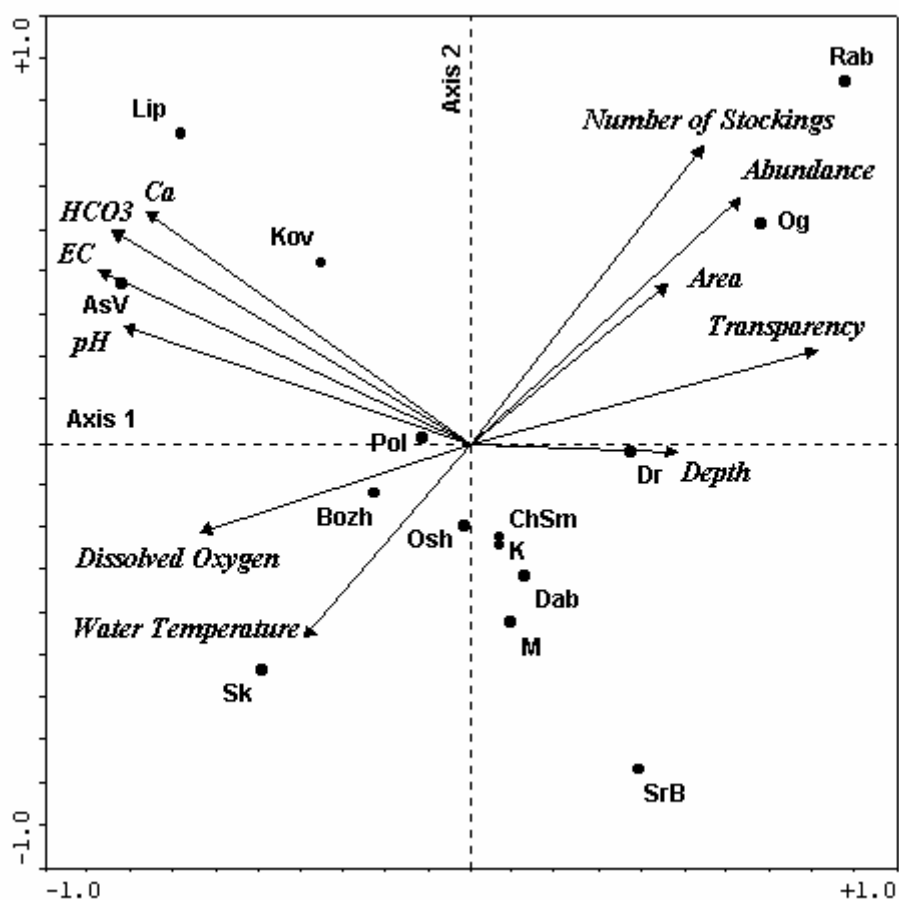


Fig. 8. Principal components analysis (PCA) correlation biplot of the data obtained in August-September 2006. For reservoir codes see Table 1.

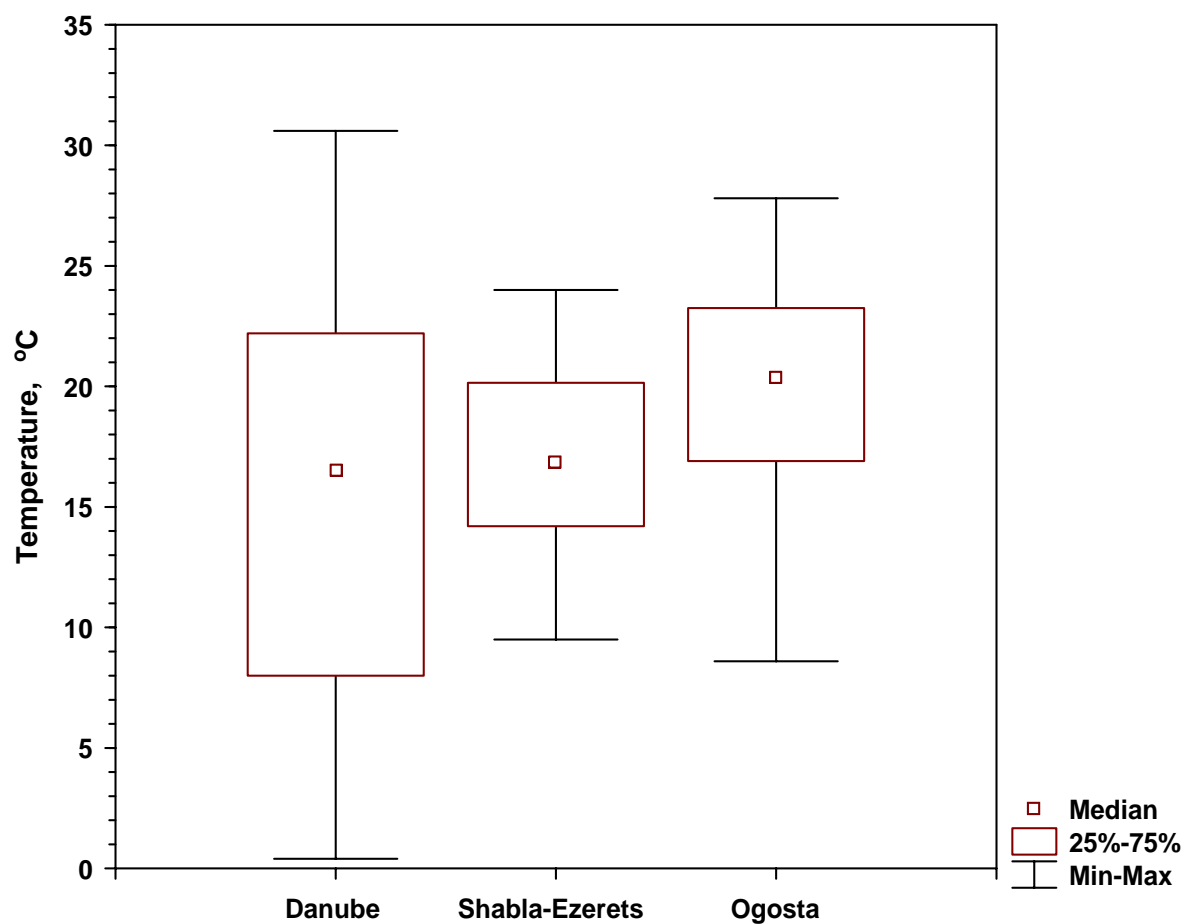


Fig. 9. Distribution of water temperature for April-September period in the Danube River (1990-2005), the Shabla-Ezerets Lake System (1992-1994) and the Ogosta Reservoir (1998-2006).

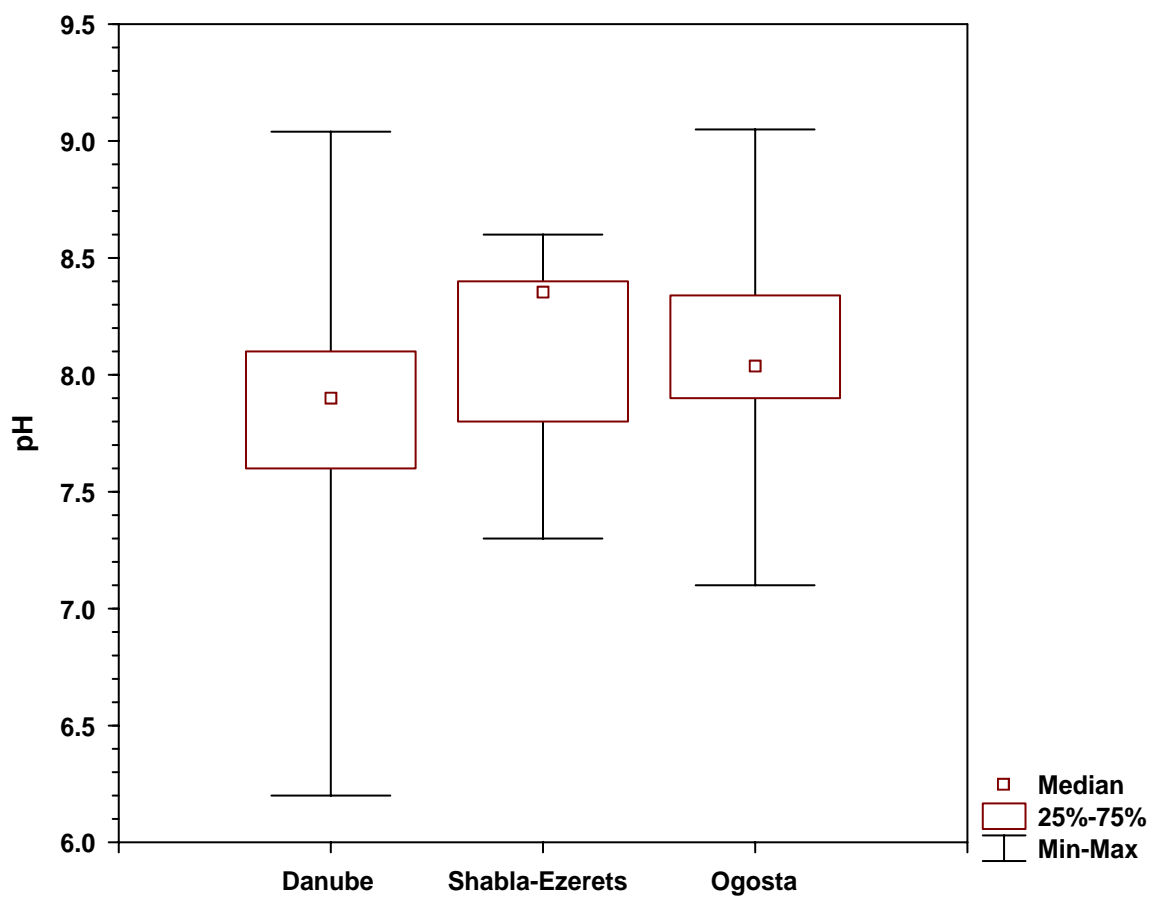


Fig. 10. Distribution of pH for April-September period in the Danube River (1954-1961, 1990-2005), the Shabla-Ezerets Lake System (1992-1994) and the Ogosta Reservoir (1998-2006).

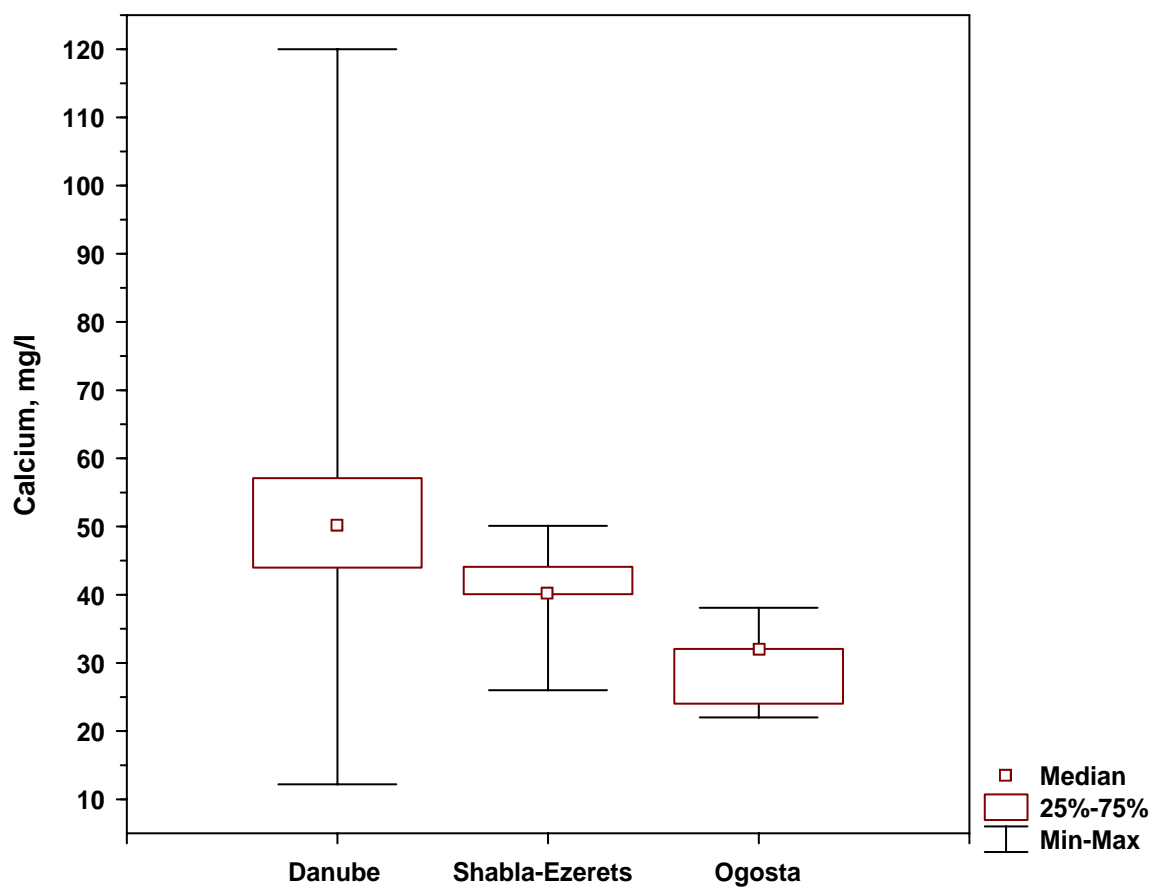


Fig. 11. Distribution of calcium concentration for April-September period in the Danube River (1954-1961, 1990-2005), the Shabla-Ezerets Lake System (1992-1994) and the Ogosta Reservoir (2005-2006).

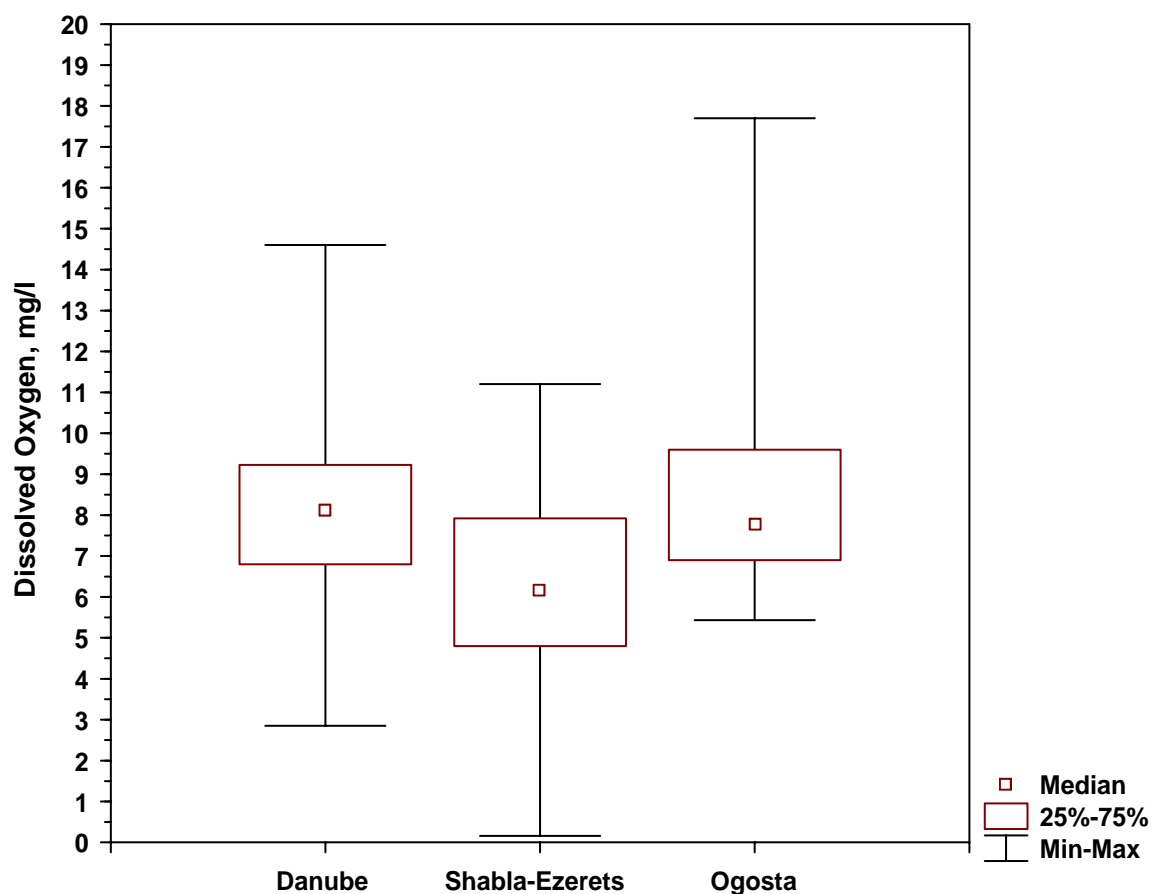


Fig. 12. Distribution of dissolved oxygen concentration for April-September period in the Danube River (1990-2005), the Shabla-Ezerets Lake System (1992-1994) and the Ogosta Reservoir (1998-2006).

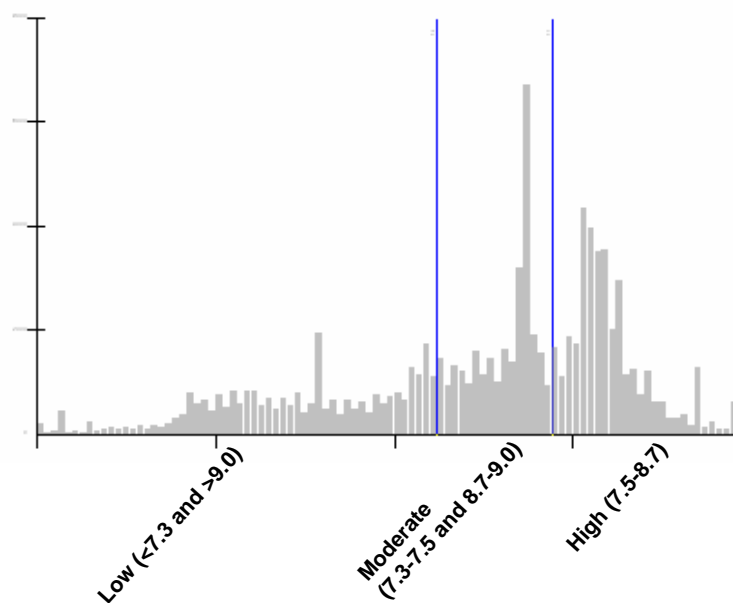


Fig. 13. Histogram of Ph values obtained after spatial interpolation procedure.

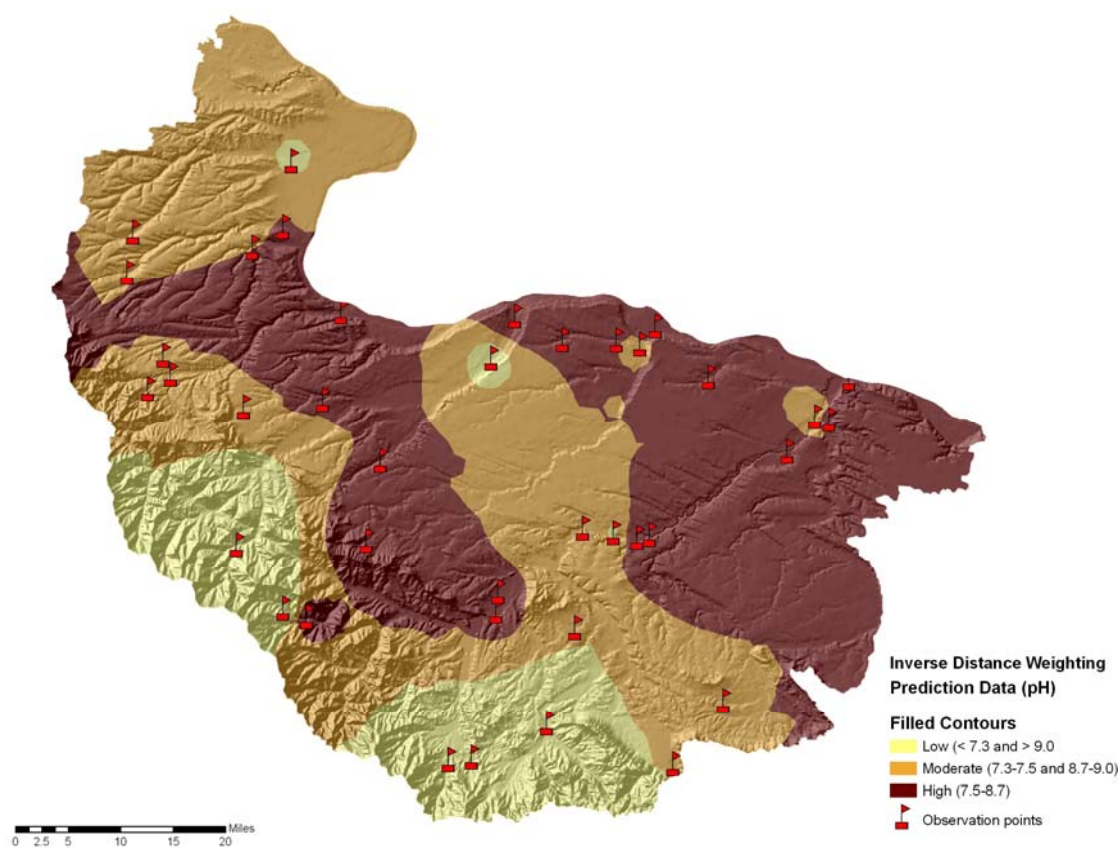


Fig. 14. Inverse distance weighting prediction data for pH in the North-West Bulgaria.

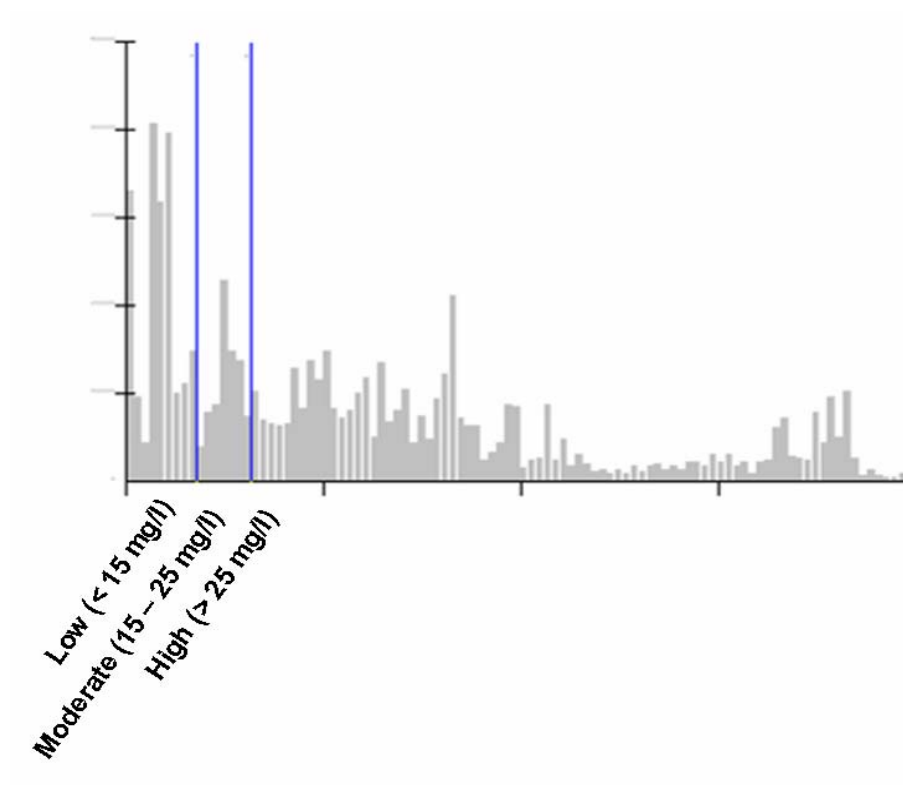


Fig. 15. Histogram of calcium values obtained after spatial interpolation procedure.

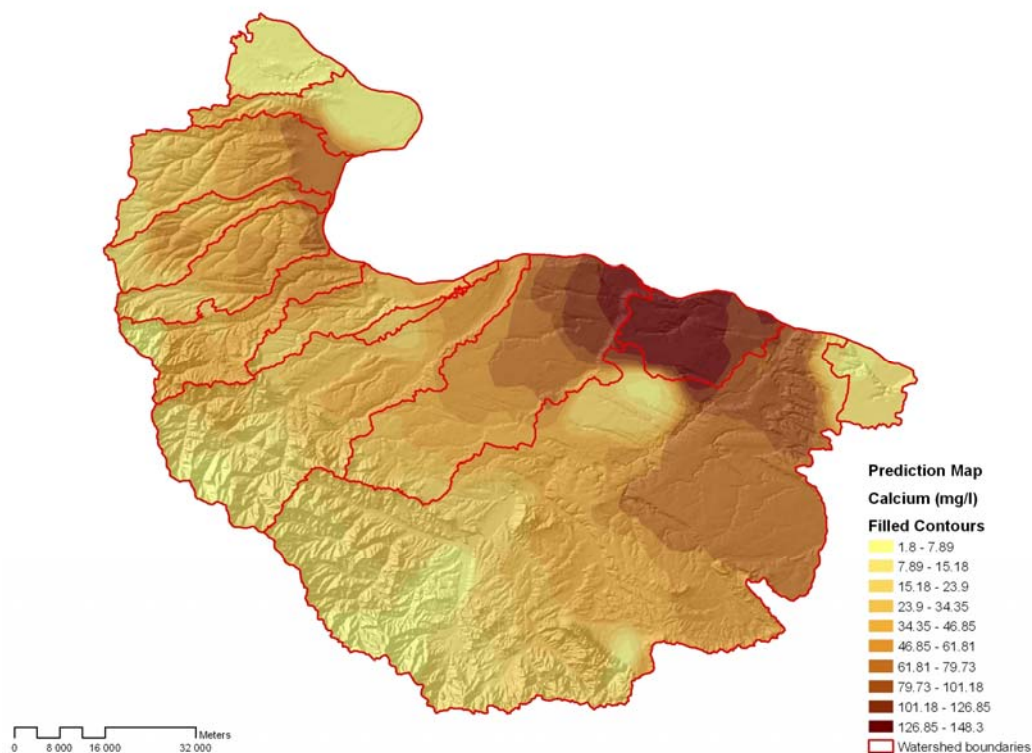


Fig. 16. Prediction map of calcium concentration in the North-West Bulgaria.

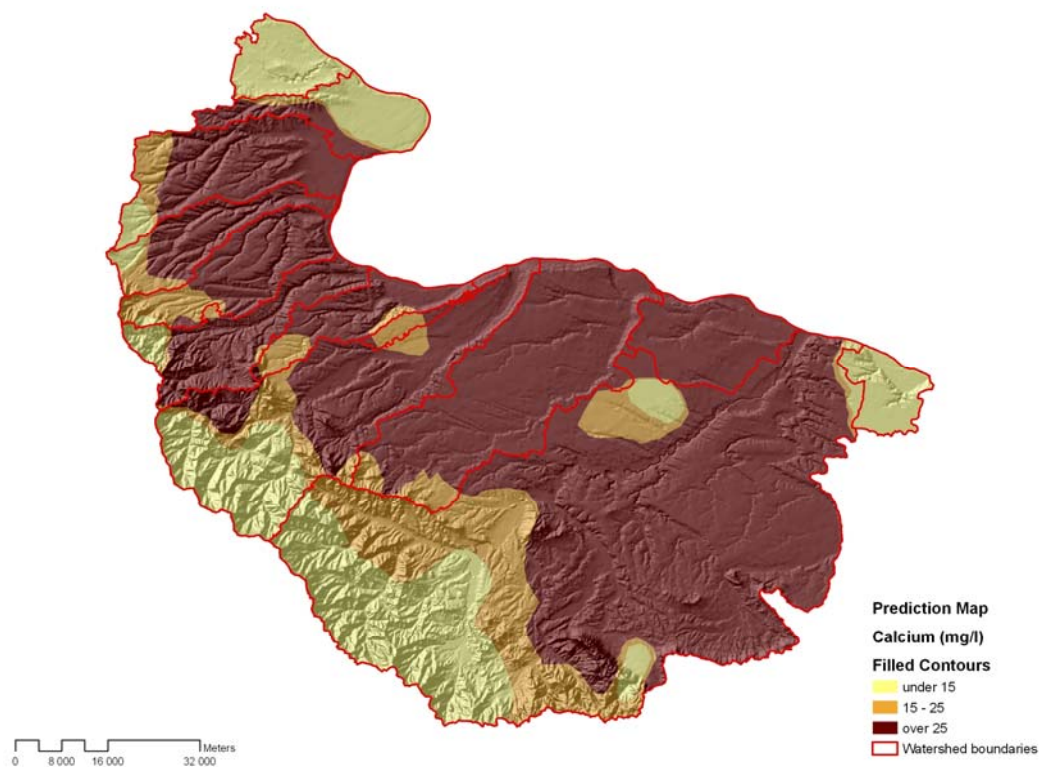


Fig. 17. Distribution of calcium concentration in the watersheds of the North-West Bulgaria.

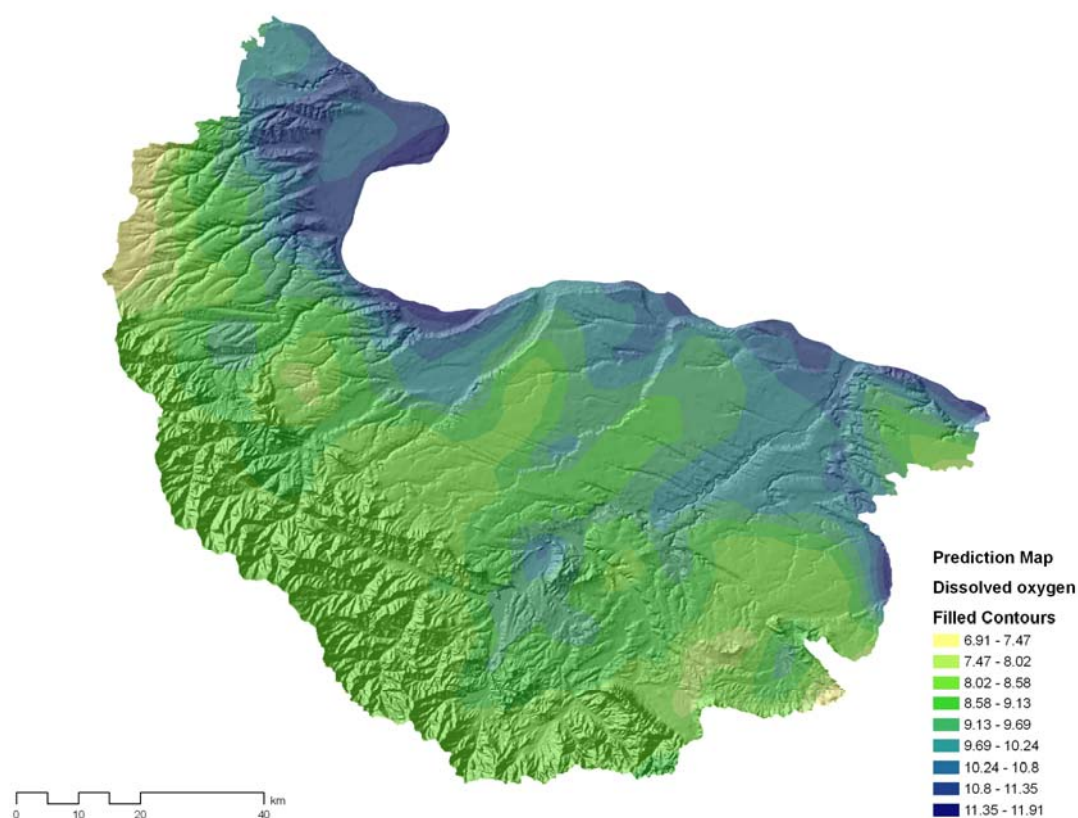


Fig. 18. Prediction map of dissolved oxygen concentration in the North-West Bulgaria.

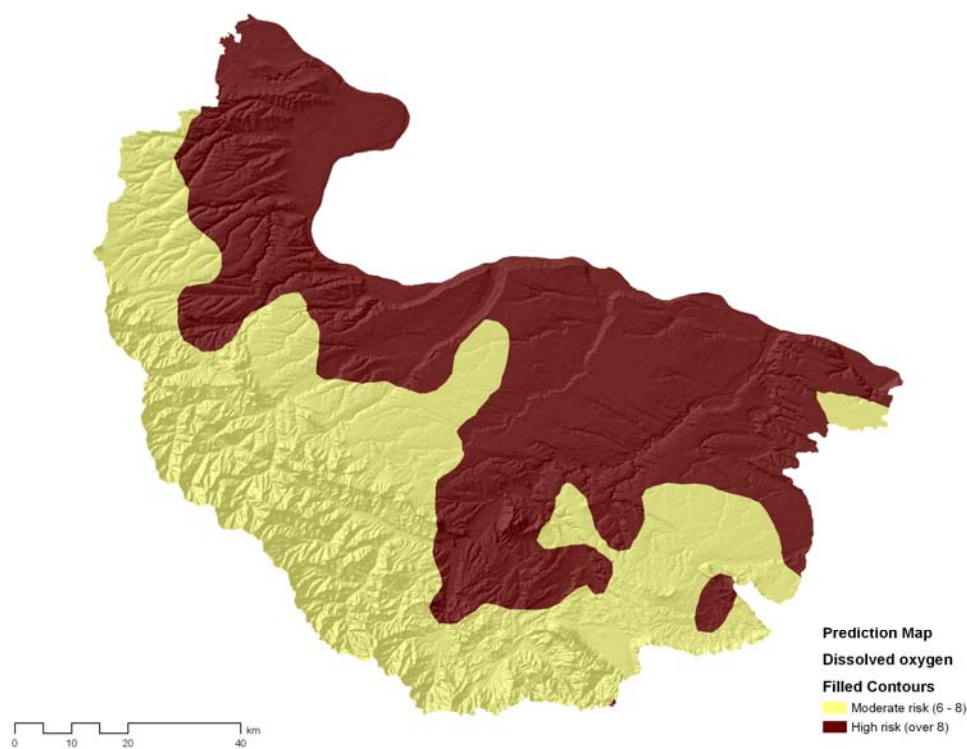


Fig. 19. Estimation of dissolved oxygen concentration in the North-West Bulgaria.

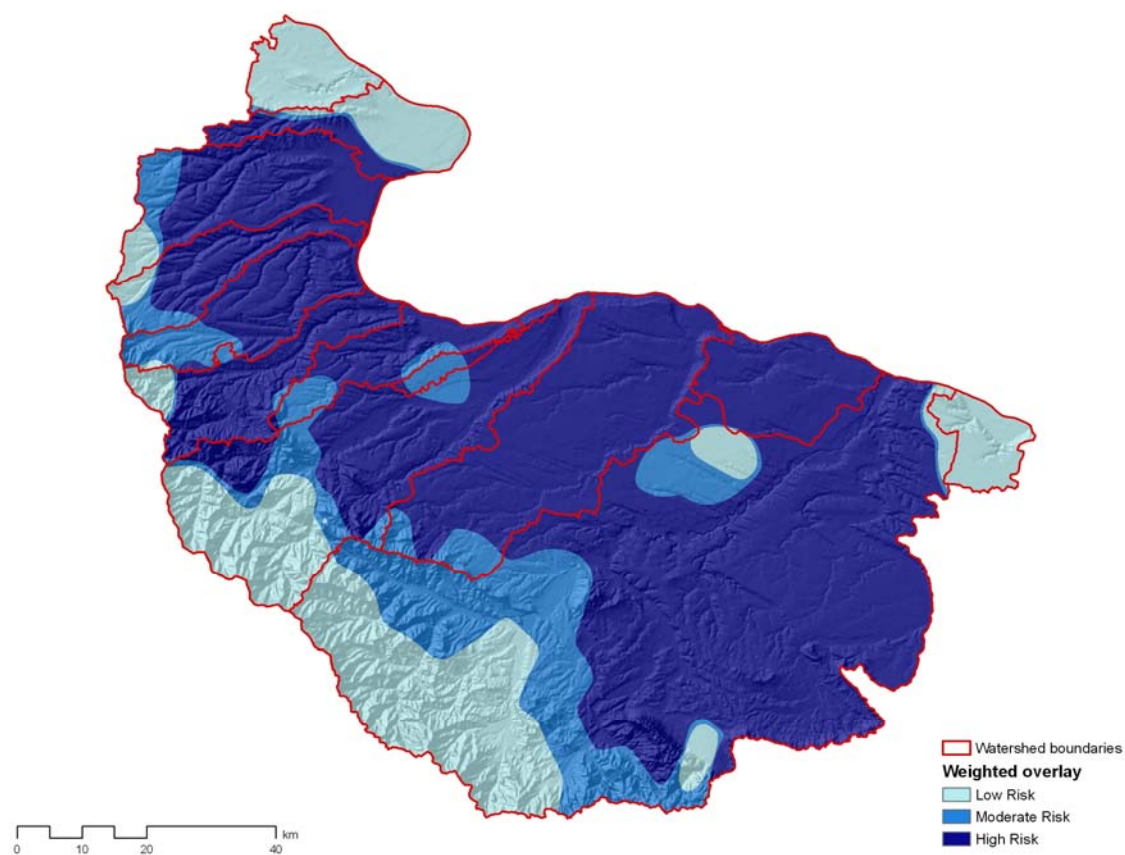


Fig. 20. Infestation potential of zebra mussel in the North-West Bulgaria.